INTEGRITY OF STORAGE MEDIA FOR CLINICAL APPLICATIONS WITH SIFT-MS INSTRUMENTS

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ABSTRACT

Tedlar™ bags are a promising medium for remote breath collection and later analysis using SIFT-MS for disease diagnosis. It is important to understand the changes in integrity of samples stored in Tedlar™ bags. However, there is little work into this problem completed to date, and thus little known about these issues. Therefore, a study into the integrity of samples stored in Tedlar™ bags and analysed using SIFT-MS was undertaken.

The sample integrity of ammonia, acetone, ethanol, isoprene and pentane, all initially at 3ppm in breath and nitrogen substrates, and stored in Tedlar™ bags was investigated. Experiments tested the effect of storage size (0.5, 1, 3L), storage time (6-48 hours), storage temperature (23°C - 25°C, 37°C), humidity (0.4 – 4.5% absolute) and inter-bag variation using triplicate bags. The SIFT-MS instrument used was LDI2 located at Christchurch Hospital. The repeatability and precision of LDI2 was established using prepared cylinder samples (0.05% absolute humidity) of acetone, pentane and ethanol tested at seven times over a 250 min time period. A generalised Cauchy distribution was used to give a combined distribution from multiple bags for the sample humidity and compound concentration.

A combined measure of the repeatability and precision, s_T, ranged between 217 – 349 ppb for ethanol, acetone and pentane. The factors affecting the repeatability and precision were both machine and compound dependant. The effect of the factors differed over time, with different precursors and compounds. No obvious effects of bag storage size on the sample integrity of pentane, isoprene, ethanol and acetone were

observed. The absolute humidity change within bag samples was linked to the volume to surface area ratio because it was more affected by permeation and condensation.

All compounds in the nitrogen substrate (except for 37°C stored acetone (NO+)) displayed decreases in sample integrity with time. All compounds in the breath substrate displayed regular losses of sample integrity, except for the 37°C and 23°C - 25°C stored ethanol (NO+) and 37°C stored ethanol (H3O+), pentane (O2+) and ammonia (H3O+, O2+). The average change of sample integrity for pentane, isoprene, ethanol and acetone ranged from 0.2 to 3.6 times the maximum s_T, while ammonia ranged from 0.9 – 10 times. All observed behaviour was reproducible.

Absolute humidity and storage temperature affected the sample integrity of acetone, ethanol and ammonia. Generally, the intra-bag variance was comparable between all storage temperatures and substrates while the inter-bag variation was affected by the absolute humidity. Only the initial and final concentrations between precursors for the 23°C - 25°C stored breath and nitrogen substrates agreed. The breath substrate samples gave erroneous values for ammonia. Permeation of compounds into the bags from the atmosphere was not significant.

The overall issues surrounding storing breath in Tedlar™ bags for analysis using SIFT-MS is not the loss of sample integrity, but the kinetics, precision and repeatability of the SIFT-MS instrument. The current kinetics are not adequate to accurately monitor acetone, isoprene, pentane, ammonia and ethanol in breath and stored in Tedlar™ bags at breath absolute humidity levels greater than 3%. Generally, the loss of sample integrity was only marginally outside the repeatability and precision of the machine.

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1 INTRODUCTION

Exhaled breath contains an assortment of compounds that can provide information about a person's physiological state. Numerous studies have found that people suffering from certain diseases have elevated concentrations of some compounds in their breath (Riely, Cohen et al. 1974; Mukhopadhyay 2004; Davies, Spanel et al. 1997; Smith and Spanel 2005; Risby 2005). Measured concentrations are outside normal ranges for healthy individuals, demonstrating the potential of chemical breath analysis to result in effective disease diagnosis. Breath tests as a diagnosis tool are attractive as they are non-invasive and enable multiple sample collection without significant risk to the patient or person collecting the sample.

The concept of using breath to provide clues as to people's physiological state was originally described by Hippocrates around 400 BC. For many years physicians have used their own sense of smell as an aid to diagnose disease. The rotting apple or urine like smell on a patient's breath was commonly used to diagnose "evil humors", which would later be called diabetes and renal failure. In 1784 Lavoisier identified that carbon dioxide is in breath. Lavoisier's discovery was the first quantitative study and identification of compounds in breath, paving the way for breath analysis. However, it was not until the 1960s and 70s that the first publications on breath analysis were

written (Jansson and Larsson 1969); (Pauling, Robinson et al. 1971; Riely, Cohen et al. 1974; Dannecker JR, Shaskan et al. 1981; Chen, Zieve et al. 1970). The reason for slow progress has been the technological inability to reliably and easily detect and quantify compounds in breath. Of all the human senses, our rapid sense of smell has only been partially replicated by science and engineering recently. As new analysis technologies are developed and the sensitivity of current techniques is increased the potential for breath test diagnosis in health also increases.

Breath is a mixture of nitrogen, oxygen, carbon dioxide, water vapor, inert gases and other volatile organic compounds (VOCs). VOCs are organic chemical compounds that are able to evaporate at normal atmospheric conditions and enter the atmosphere. The VOC's in breath are a mixture of as many as 200 different compounds (Phillips, Greenberg et al. 1994) from a possible 3481 that have been identified to date (Phillips, Herrera et al. 1999). The VOC's found in breath are of particular interest to disease diagnosis because many originate predominantly from the blood.

Many VOCs occur in breath primarily due to the gas exchange that occurs between the blood and inspired air. The gas exchange occurs in the lungs where the ability to exchange oxygen for carbon dioxide is essential for life. The lungs are part of the respiratory system along with the nose, mouth and trachea as shown in Figure 1.1.

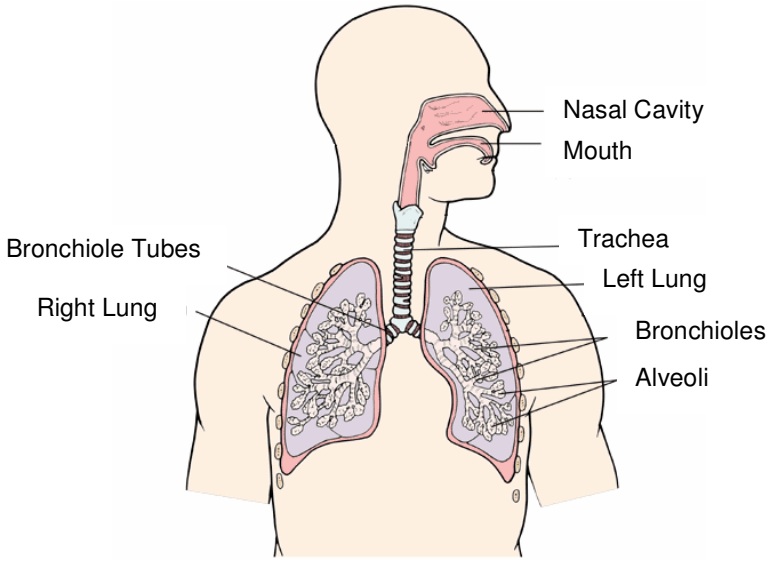

Figure 1.1: Respiratory system (McKesson 2005)

Gas exchange occurs in a region of the lungs called the alveolus as shown in Figure 1.1. The alveoli are attached to the final branches of the bronchioles, which end as sacks called alveoli (singular = alveolus) as shown in Figure 1.2. Each single alveolus is covered in pulmonary capillaries. The gas exchange occurs between the pulmonary capillaries and inspired air in the alveolus, as shown in Figure 1.3. Various biological processes involving the tissue surfaces of the nose, mouth and trachea are another source of VOC's in the breath.

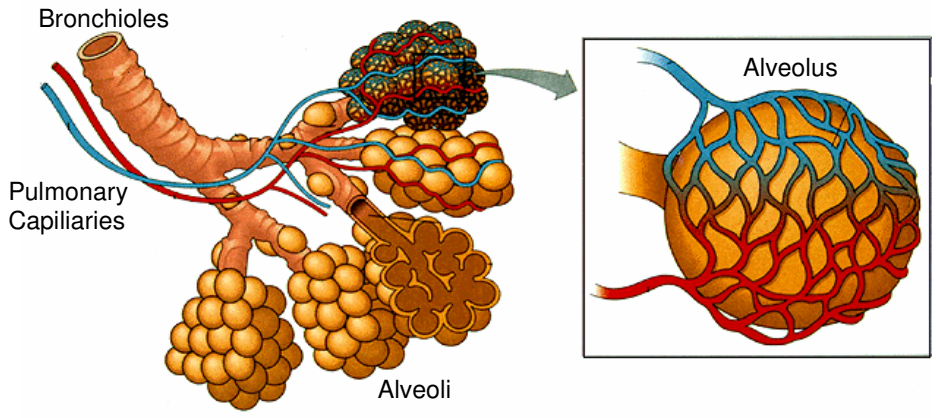

Figure 1.2: Alveolar (Tamarakin 13/3/2006)

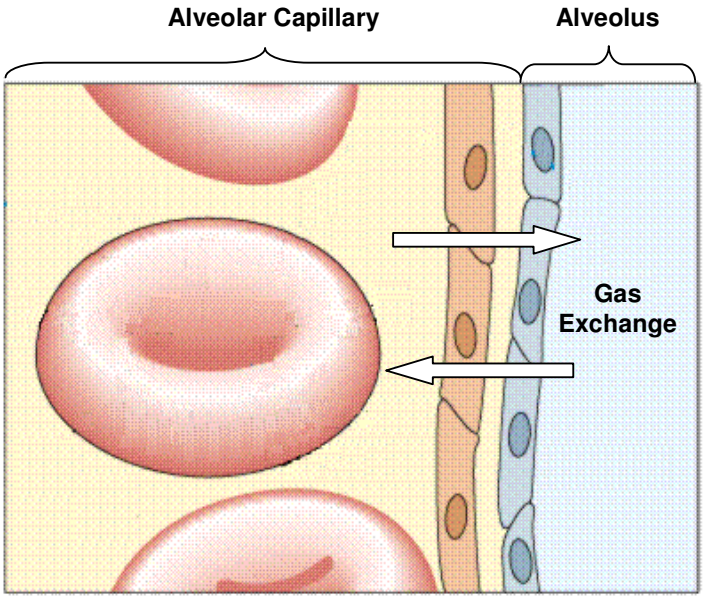

Figure 1.3: Gas Exchange across alveolar capillary and an alveolus (Purves 2004)

The diffusion of gases is governed by the concentration gradients across the alveolar capillaries and alveoli. This is therefore a two way process, where compounds in the air, which are at a greater concentration than the blood, move into the blood and vice versa. The expired breath therefore contains molecules from the inspired air and molecules that were present in the blood.

VOC's within the body and blood arise from a number of origins. The origins fall into two groups, exogenous and endogenous. Endogenous origins are from within the body, while exogenous origins can come from any number of environmental sources external to the body. As shown in Figure 1.4, these sources all contribute to the VOC's found in breath (Phillips, Greenberg et al. 1994).

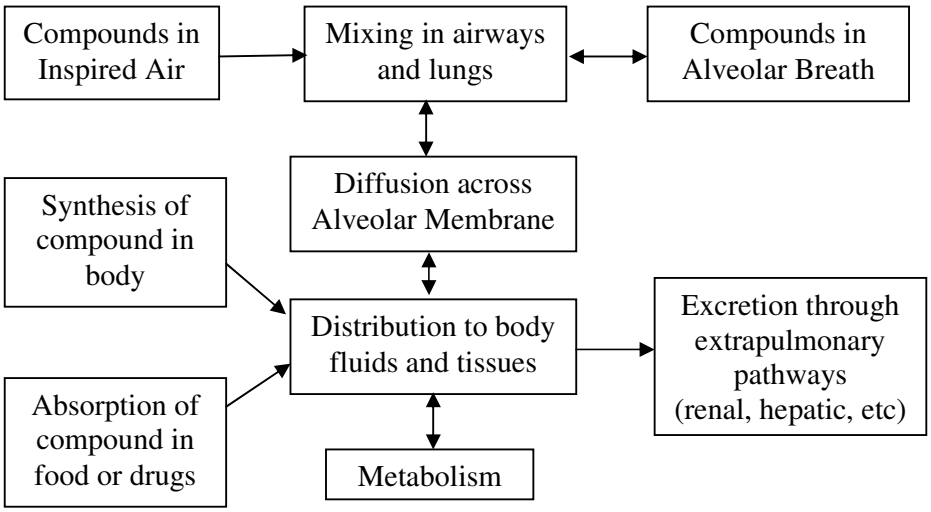

Figure 1.4: Sources of VOC's to breath (Phillips, Greenberg et al. 1994)

It is important to understand the origin of VOCs if they are to be used for disease diagnosis and there is much debate over the origins of some VOCs. Another issue is how VOCs present in the ambient, inspired air should be managed. Some researchers feel that the environmental VOCs should be subtracted from exhaled air samples (Phillips, Greenberg et al. 1994; Phillips 2005), while others ignore them. The answer to background environment VOC's requires more research to answer, but is something that must be resolved before breath testing for specific clinical uses can proceed.

1.1 COMMON VOC'S AND NORMAL LEVELS

Some of the 3481 VOC's that have been identified in breath occur more commonly and at generally higher concentrations than others (Phillips, Herrera et al. 1999). The most common and abundant breath metabolites are ammonia, acetone, ethanol and isoprene (Gelmont, Stein et al. 1981; Manolis 1983; Smith, Spanel et al. 1999; Diskin, Spanel et al. 2003; Fenske and Paulson 1999). The mean concentrations in ppb of

each metabolite are ammonia, 422–2389; acetone, 293–2000; isoprene, 55–600; ethanol, 27–1000 (Diskin, Spanel et al. 2003; Baumbach, Vautz et al. 2005). Pentane is also a common breath compound which is at relatively low levels, 6-21 ppb in breath (Mendis, Sobotka et al. 1994).

1.2 ENDOGENOUS SOURCES OF COMMON VOC'S

These common metabolites come from different endogenous sources and are indicative of many diseases.

Pentane is mainly linked to lipid per-oxidation (Frankel 1983; Wade and van Rij 1985; Weitz, Birnbaum et al. 1991; Kneepkens, Lepage et al. 1994; Risby and Sehnert 1999). Pentane levels are also increased in patients with asthma (Olopade, Zakkar et al. 1997) obstructive sleep apnoea (Olopade, Christon et al. 1997) pneumonia (Schubert, Muller et al. 1998), ARDS (Schubert, Miekisch et al. 2003), acute rejection of transplanted hearts (Holt, Johnston et al. 1994), breast cancer and schizophrenia (Phillips, Herrera et al. 1999). Acetone is linked to dextrose metabolism which is an indication of diabetes mellitis (Lebovitz 1995; Nelson, Lagesson et al. 1998). Isoprene is associated with cholesterol metabolism (Stone, Besse et al. 1993) and critically ill patients (Schubert, Muller et al. 1998), while ammonia is associated with liver disease (Chen, Zieve et al. 1970), renal failure (Davies, Spanel et al. 1997) and chronic active gastritis and ulcers in the stomach and duodenum (Penault, Spanel et al. 2005). Ethanol is principally produced by the action of gut bacteria on carbohydrates (Owades 2000) and apart from its association with law enforcement with blood alcohol levels, it may also be important in renal failure (Smith and Spanel 1996). All of these conditions mentioned would benefit from rapid and easy diagnosis.

1.3 COMMON BREATH TESTS

It has been over 200 years since Lavoisier first quantified CO_2 as a breath analyte and 30 years since the first publications, but breath test diagnosis is still not commonplace. Currently there are just seven approved and widely used breath tests (Risby 2005):

- Breath Carbon Dioxide Test for capnography
- Breath Carbon Monoxide Test for neonatal jaundice
- Breath Ethanol Test for blood ethanol (law enforcement)
- Breath Hydrogen Test to detect disaccharidase deficiency, gastrointestinal transit time, bacterial overgrowth, intestinal statis
- Breath Nitric Oxide Test for asthma and its therapy
- Breath Test for detection of heart transplant rejection
- Urea Breath Test for detection of *Helicobacter pylori* infection

The significant clinical range of the common breath tests indicates the broad potential of the approach.

1.4 REQUIREMENTS OF BREATH SAMPLING

Sampling of biological fluids, such as blood, is well developed, understood and widely implemented. However the practical ability to collect and analyse breath is still lacking. As breath is a complex mixture, accurate quantification for clinical diagnosis has been particularly challenging. Breath testing has a number of requirements and problems that must be solved to ensure regular clinical use (Cao and Duan 2006). Developments need to be made in all areas from sampling, analyzing to diagnosis. More importantly, before useful research into VOC markers indicative of specific

disease states can be done, the sampling and analysis of breath samples must be improved, validated and standardised.

Sampling involves collection from patients and possibly the transport of samples. The next step, analysis, is the technique used to identify and quantify the compounds in the sample. These two steps are closely linked, as the sampling procedure must deal with a sample in a way which makes it compatible for testing using the analysis technique. The recent development of Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) has now made it possible to analyse breath samples in real time, providing a unique opportunity to practically study breath diagnosis in clinical applications.

1.5 SIFT-MS

Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) was originally conceived from flowing afterglow instruments developed in the 1960s. The flowing afterglow technique uses a flowing gas of hydrated hydronium ions in inert helium or argon gas which flow down a tube where they react. The ions react with water molecules in a sample and the products from these reactions are measured by a quadrupole mass spectrometer. The major problem of the flowing afterglow technique was that the ionisation region was part of the flow tube which complicated analysis. This problem was overcome in 1976 by the development of the Selected Ion Flow Tube instrument (SIFT) (Adams and Smith 1976). Therefore, SIFT differed in having a separate ionisation region and allowing only selected ions to enter the reaction tube.

The first application of SIFT was to create kinetic data on gas phase ion-neutral reactions (Smith and Adams 1987). This was important in studying molecules formed in interstellar clouds (Smith and Spanel 1992). After thirty years, SIFT has been used

to build up a large kinetic database of ion-neutral reactions containing the products and rate constants of reactions. In the 1990's it was realised that if the kinetics of an ion-neutral reaction were known then the analysis could be reversed (Smith and Spanel 1996). The reacting sample could therefore be identified and its concentration found. These realisations lead to the development of SIFT-MS.

For many years SIFT-MS instruments were research tools and had to be custom built and operated. The research instruments were large and weighed in excess of 4 tonnes with footprints of 2-4 meters in length. This made them unsuitable for commercial applications such as airports, ports, factories or hospitals. Recently, commercially available instruments have become available which have reduced the practical problems associated with earlier instrumentation.

1.6 SUMMARY

People have used their own sense of smell as a diagnostic tool since medicine began. The odour in breath originates from an assortment of compounds which arise primarily from gas exchange between inhaled air and blood. Elevated levels of certain VOC's in the breath can be used as an indication of certain diseases or a person's physiological state. Breath testing however is still not routine due to a number of problems associated with sampling and analysis which must be addressed. Using the SIFT-MS technique it is now possible for accurate quantification of complex mixtures, such as breath, providing a unique opportunity to practically apply breath test diagnosis.

The recent commercialisation of SIFT-MS by Syft Technologies and use of SIFT-MS at the Christchurch Hospital in New Zealand has created a need to develop both indirect and direct sampling devices and procedures for breath testing. Specific requirements are:

- development of sampling equipment to interface the SIFT-MS Lab Demonstration Unit 2 (LDI2) instrument which enables direct sampling of breath.
- development of sampling equipment to remotely collect breath samples for later analysis using SIFT-MS.

This thesis covers aspects related to the development of sampling equipment to remotely collect breath samples. The development of the physical device, software and hardware are covered by Ketan Lad in his thesis. This thesis examines all the relevant aspects of the collection medium used to store collected breath and quantifying its variability and suitability as a sampling method for breath and later analysis using SIFT-MS.

This thesis is out lined as follows:

- Detailed description of the SIFT-MS technique and how it quantifies whole air samples including possible applications.
- Discussion of possible collection medium to store remotely collected breath including a review of methods used in current remote breath collection systems.

- Discussion of the factors affecting sample integrity, including choice of tested compounds and storage conditions.
- Explanation of the experimental method used to establish the integrity of samples stored in the chosen collection medium and the repeatability and precision of the SIFT-MS instrument used to measure concentrations within the collection medium.
- Explanation of the statistical analysis method used to indicate the variability of the collection medium and the machine repeatability and precision. The settings used by the SIFT-MS instrument to determine compound concentrations are also discussed.
- Results on the determined machine repeatability and precision, and the chosen collection medium variability
- Discussion of the reasons for the observed machine repeatability and precision and the chosen collection medium variability.
- Conclusion of the work including a discussion on the suitability of the chosen collection medium as a sampling method for breath and later analysis using SIFT-MS. Recommendations are finally made for any future work.

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2 SIFT-MS TECHNOLOGY

SIFT-MS is a mass spectrometric method that allows simultaneous quantitative analyses of several trace gases in air and exhaled breath. It has resolution to ppb levels and returns results in less than a second (D. Smith & Spanel, 1996; C. G. Freeman & M. J. McEwan, 2002; C. G. Freeman & M. J. McEwan, 2002). These attributes are all unique to this technology.

SIFT-MS works by using positively charged precursor ions to react with VOC's in samples via chemical ionization. Chemical ionization is the formation of a new ion by the reaction of a neutral species with an ion. An ion is an atom or group of atoms with a net electric charge while a neutral species is one without a charge.

Precursor ions are selected to react and form particular product ions. Because the reacting species is in air or breath, the precursor ion must not react with the main compounds found in air or breath (N_2, O_2, CO_2, Ar). As a result, H_3O^+, O_2^+, and NO^+ ions are typically used (C. G. Freeman & M. J. McEwan, 2002).

The reaction of the precursor ions with the neutral species forms product ions, that can be detected and used to indicate the VOC's present in the sample. Knowing the rates at which these reactions occur and the products formed enables the reacting chemical

and its concentration to be identified (D. Smith & Spanel, 1996). Because the introduced sample is a substance or chemical constituent that is undergoing analysis via this reaction with the precursor it is often referred to as the analyte.

The process that a SIFT-MS uses to identify and quantify analytes can be divided into five fundamental steps and is illustrated in Figure 2.1:

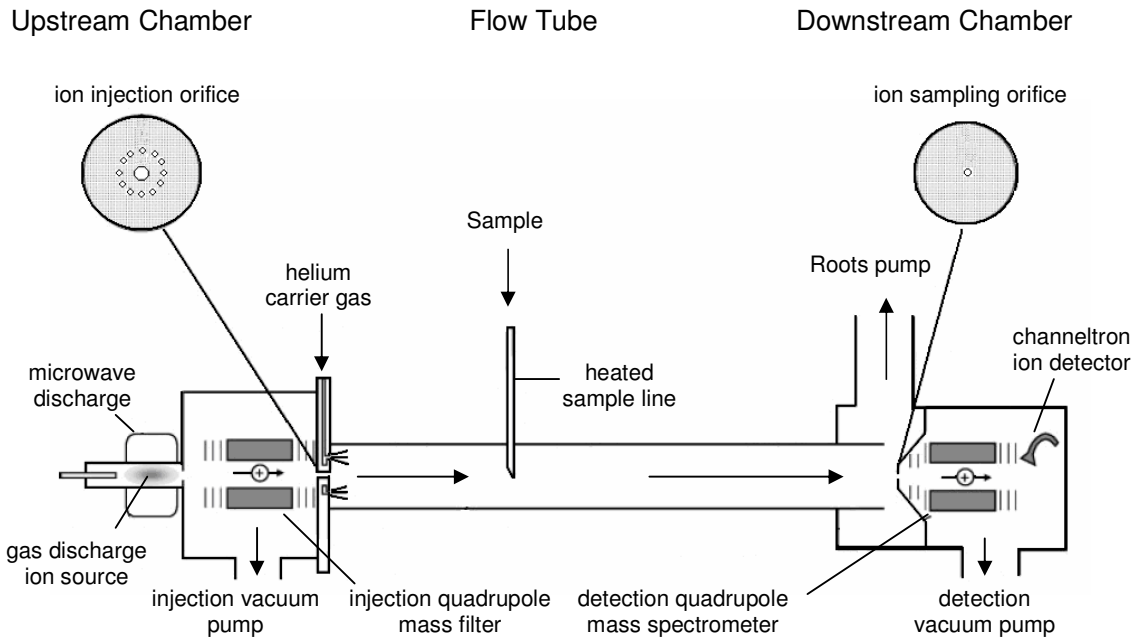

Figure 2.1: SIFT-MS (D Smith & Spanel, 2005)

[1] Creation of Precursor Ions:

Precursor ions are generated by passing water vapour or air through a microwave discharge. This process produces H_3O^+, O_2^+, and NO^+ precursors.

[2] Precursor Selection:

The precursor ions are drawn and focused through an upstream chamber where a quadrupole mass filter is used to select the required precursor ion. A quadrupole mass

filter selects ions based on their mass/charge ratio (m/z) and transmits only the ion of choice. The quadrupole consists of four parallel metal rods. A voltage frequency is applied between each opposing rod pair and superimposed with a direct current voltage. Ions travel down the quadrupole between the rods. Only ions of a certain m/z pass through for a given ratio of voltages. Because of these voltages selected, the other ions have unstable oscillations and collide with the rods. This method allows selection of a particular ion.

The selected precursor ion passes though a Venturi orifice (~1 mm diameter) and into the flow tube containing a flowing inert carrier gas. The carrier gas is usually helium (primary flow) at a pressure of 0.5 torr and flowing at a velocity of $100ms^{-1}$. Argon (secondary flow) is also used to improve the diffusion properties of the ion stream. The inert carrier gas entrains the precursor ions and sample, providing a region of known conditions. It is in this region that reaction between the sample VOCs and precursor ions occurs, creating the material for detection of VOCs from the reaction products.

[3] Reaction of Precursor with Sample:

The selected precursor ions in the flow tube react with VOCs from the sample to form product ions. The sample is drawn through a capillary which has a diameter of 1.6 mm. The capillary minimises the surface area exposed to the sample and thus loss due to surface adsorption. The flow rate through the capillary is created by the pressure difference between the atmosphere and flow tube. The flow rate is affected by the length of the capillary and its diameter, which are both carefully selected and calibrated.

[4] Product Selection:

A representative portion of the product ions pass through a 0.3 mm diameter orifice at the downstream end of the flow tube and into a second, differentially-pumped quadrupole, that filters the ions according to mass. The carrier gas is prevented from entering by the quadrupole, which deflects it away from the centre of the orifice, thus separating it from the sample to be analysed.

[5] Measurement of Products:

The selected product ions pass to the channeltron particle multiplier/detector where they are counted. The channeltron multiplier/detector is a compact form of an electron multiplier detector. An electron multiplier detector multiplies charge through the impact of ions on its surface. The impact emits electrons which accelerate into a metal plate which causes the emission of more electrons. The process is repeated until the desired multiplication of the original ions has occurred.

Overall it should be noted that the upstream chamber, flow tube, and downstream chamber all operate under vacuum pressure. Vacuum pressure creates pressure differentials to drive flow along the flowtube and introduce sample through the capillary (D Smith & Spanel, 2005). Currently, Syft Technologies SIFT-MS instruments require two main pumps to create these conditions.

2.1 MODES OF OPERATION

SIFT-MS carries out two main types of analysis, SIM (Selected Ion Mode) scans and mass scans. SIM scans are analysis specific to target analytes based on the detection of specific, known product masses. Only the counts of the precursor and product masses are monitored. The downstream mass spectrometer switches between

monitoring the products ions and precursor ions, spending a short specified time (25-50 ms) monitoring each. SIM scans thus give the concentration of the analyte in real time. A typical SIM scan is shown in Figure 2.2.

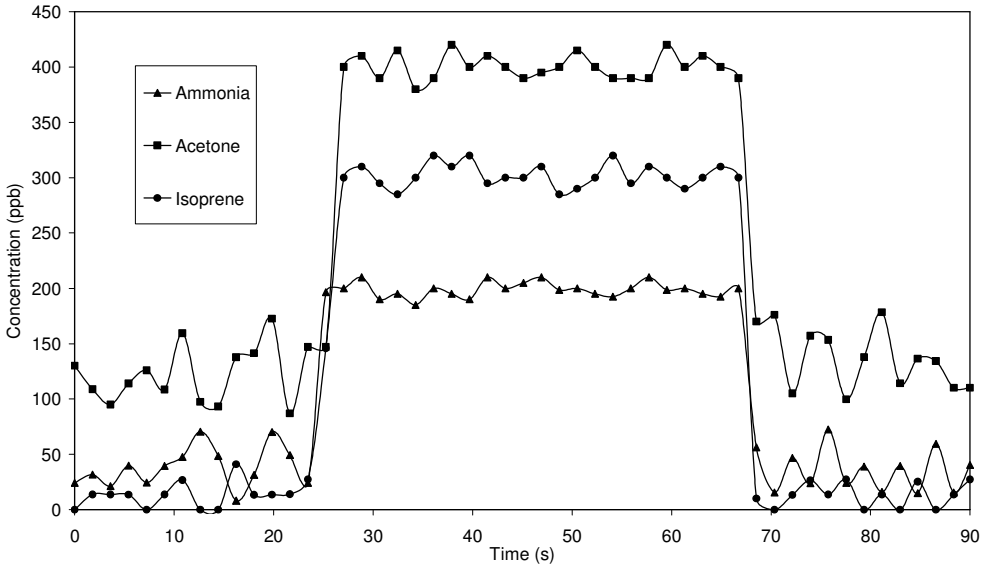

Figure 2.2: Typical SIM scan

Mass scans are scans over the instrument mass detection range (10 to 300 atomic mass units (amu)) with a chosen precursor. It is preformed by scanning the quadrupole over a selected mass-charge ratio (m/z) range, while a sample is introduced. Mass scans give the count rate calculated from knowing the number of counts as given by the channeltron particle multiplier and sampling time for each mass. Peaks in the mass spectra are then related to the trace gases present in the sample. An example would be to use a mass scan to identify the product masses of a reaction and then use a SIM scan to monitor the concentration of the sample in real time, once significant mass peaks were identified. A typical mass scan is shown in Figure 2.3.

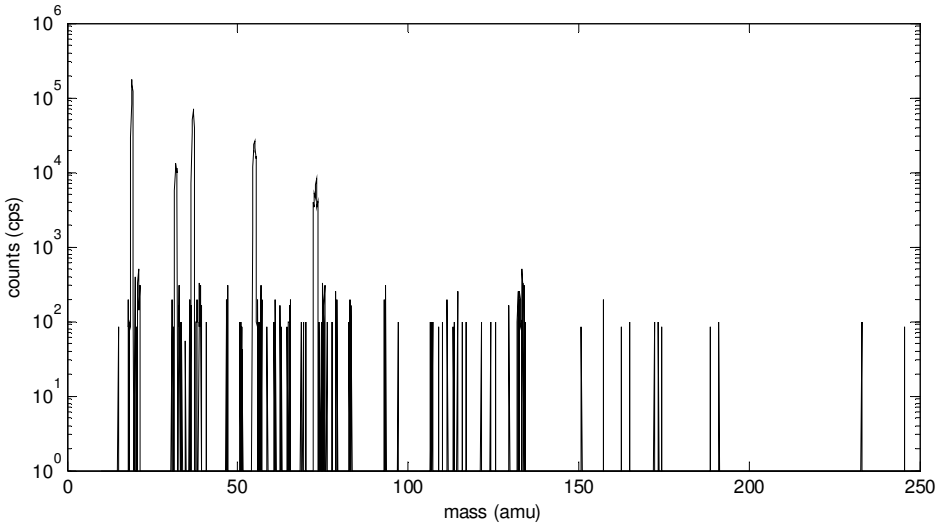

Figure 2.3: Typical mass scan

2.2 COMPARISON WITH GAS CHROMATOGRAPHY MASS SPECTROMETRY (GC- MS)

GC-MS is an analytical technique used for breath scanning and analysis. Hence is represents a gold standard, of sorts, for comparing with SIFT-MS technology. GCMS is the combination of two techniques, gas chromatography and mass spectroscopy.

Gas chromatography separates the components of a mixture, and mass spectroscopy detects and characterizes each of the components. In gas chromatography the sample mixture is separated by mixing it with an inert gas and passing it through a chemical that selectively attracts components in a sample mixture contained in a column. Each compound in the mixture interacts at a different rate, with those interacting the fastest exiting the column first. As the compounds are separated they are bombarded by electrons, breaking the compounds into fragments. The fragments are then filtered using a quadrapole and passed to a detector that provides the mass spectroscopy part of the system.

SIFT-MS differs significantly from GC-MS in key aspects. The greatest difference is the time required for analysis and sample preparation. Due to the need to separate the sample, GC-MS takes considerably more time (10-45 min) than SIFT-MS (<5s) for sample analysis. GC-MS requires that samples are transferred to evacuated chambers so that they can be purged with an inert gas before entering the machine (10-30mins). SIFT-MS does not require any such sample preparation. GC-MS is also more labour intensive then SIFT-MS when analysing whole air samples such as breath. Because the column used by GC-MS to separate compounds is not universally applicable to all compounds, a number of different columns may be required to analyse the wide range of compounds found in whole air samples. It is therefore more labour intensive than SIFT-MS which requires no separation. GC-MS must also be calibrated using standards spanning the required sample range, which takes up to half an hour prior to testing. In contrast, SIFT-MS is not calibrated, but needs to be validated against standard gas mixtures taking approximately 1min using pre-made known test samples. Both machines are comparable based on detection limits, accuracy and operating costs. Hence the SIFT-MS approach offers operational potentially reduced likelihood of contamination as well as greater speed and flexibility in clinical use.

2.3 FINDING REACTION RATE COEFFICIENTS - KINETICS

The reaction rate coefficient describes the speed at which reactions occur and has units of cubic centimetres per molecule per second. The rate coefficient links the rate of product formation with reactants.

$$A + B \xrightarrow{k} C + D \qquad (2.1)$$

Rate coefficients change considerably with temperature and hence the controlled environment within the SIFT-MS flow tube must maintain constant temperature. Reaction rate coefficients have been identified for many reactions of many different reactants and as discussed previously, was the initial reason for the development of SIFT-MS.

As SIFT-MS uses reaction rate coefficients and not empirical factors to find the concentration of the reactants after measuring the products it does not need to be calibrated. However, the determination of the reaction rate coefficient and identification of products, including their distribution, could be considered a calibration method. The reaction rate coefficients can be found using two methods, absolute methods and relative methods (Syft, 2005a).

[1] Absolute Methods:

Absolute methods involve determination of the actual reaction rate coefficients for reactions and are done experimentally. First the carrier gas flow rate is defined:

$$\Phi_c = \Phi_{He} + \Phi_{Ar} \qquad (2.2)$$

Where:

Φ_{He} = Helium Gas Flow Rate (torr Ls^{-1})

Φ_{Ar} = Argon Gas Flow Rate (torr Ls^{-1})

Total flow rate in flow tube is defined:

$$\Phi_{Total} = \Phi_C + \Phi_S \qquad (2.3)$$

Where:

Φ_{Total} = total flow in flow tube (torr Ls^{-1})

Φ_S = sample flow through capillary (torr Ls^{-1})

Hence the gas velocity in the flow tube (Syft, 2005a; D Smith & Spanel, 2005) is defined:

$$v_g = \frac{\Phi_{Total} 4 T_g}{p_g \pi d_t^{\,2} T_{ref}} \qquad (2.4)$$

Where:

T_g = flow tube temperature (K)

p_g = flow tube pressure (torr)

d_t = flow tube diameter (m)

T_{ref} = reference temperature for rate coefficients (273 K)

Next, ion velocity (D Smith & Spanel, 2005; Syft, 2005a; D Smith & Adams, 1987) can be defined:

$$v_i = 1.5 \times v_g \qquad (2.5)$$

Where the coefficient 1.5 is an axial correction factor. The axial correction factor accounts for the fully developed velocity profile in the flow tube that results in a gas velocity along the centre of the flow tube of 1.5 times the bulk velocity in the flow

tube. This centre velocity is taken as the ion velocity as ions can only enter the quadrapole through the orifice plate.

Reaction time is then defined (D Smith & Spanel, 2005):

$$t_r = \frac{l - \varepsilon}{v_i} \qquad (2.6)$$

Where:

l = reaction length which is the distance from sample inlet port to downstream sampling orifice (m)

ε = end correction to account for finite mixing distance of reactant gas into carrier gas (m) (D Smith & Adams, 1987)

The number density [A] of reactant molecules in the flow tube can be found from knowing the flow of the sample, Φ_S and carrier gas, Φ_C , (D Smith & Spanel, 2005).

$$[A] = \frac{\Phi_S}{\Phi_{Total}} \frac{p_g}{k_b T_g} \qquad (2.7)$$

The number density of precursor ions, N_i, in the flow tube decays with time when a sample is introduced. The decay is affected by diffusive losses to the walls of the flow tube and also by reactions with the introduced sample. Diffusive losses can be described using two constants that are characteristics of the flow tube geometry, a diffusion coefficient, D_i, and diffusion length, Λ. The decay of the number density

of precursor ions in the flow tube can therefore be represented as a first order differential equation (D Smith & Spanel, 2005).

$$\frac{dN_i}{dt} = -N_i \frac{D_i}{\Lambda^2} - N_i k[A] \qquad (2.8)$$

Integrating Equation 2.8 gives a relation between the count rate of the precursor when sample is present, I, and the count rate of precursor when no sample was present, I_0 (Syft, 2004, 2005b). It can therefore be used to link the concentrations of precursor and products with time.

$$I = I_0 e^{-k[A]t} \qquad (2.9)$$

Where:

I = the ion concentration at time (cs^{-1})

I_0 = the ion concentration at time zero

k = the rate coefficient

$[A]$ = the concentration of the neutral analyte

t = the reaction time

Equation 2.9 can be rearranged to give the reaction rate coefficient (Syft, 2004):

$$k = -\frac{\ln\left(\frac{I}{I_0}\right)}{[A]t_r} \qquad (2.10)$$

The only unknowns in Equation 2.10 are I and I_0. These unkowns are measured during an experiment by recording the precursor counts before and after introducing a pure VOC sample into the flow tube. This task is normally done by keeping the number of precursor ions constant and varying the flow of the neutral ion (sample). The slope of the semi-logarithmic decay plot of precursor ion intensity against neutral flow gives the rate coefficient, *k*, for that reaction.

[2] Relative Methods:

Relative methods involve determining one rate coefficient relative to another rate coefficient. Based on fundamental theory the reaction of the H_3O^+ precursor with a neutral ion, for example B to give products BH^+ occurs at the collision limiting rate (Bohme, 1975; Bouchoux, Salpin, & Leblanc, 1996). The collision limiting rate is a measure of how strongly the precursor ion and reactant molecule are attracted to each other and has units of cubic centimetres per molecule per second (cm^3 $molecule^{-1}s^{-1}$ or more commonly cm^3s^{-1}). The collision rate coefficient is an upper limit to the experimentally determined rate coefficient, *k*. It is calculated using physical properties of the reactant gas, such as its molar mass (g mol^{-1}), dipole moment in Debye (D) and polarisability (α) in cubic Angstroms ($Å^3$). The assumption that these reactions occur at the calculable collision limiting rate allows it to be used as the relative kinetic data to find collision rates for O_2^+ and NO^+.

To use H_3O^+ as relative data all three precursors (O_2^+, NO^+ and H_3O^+) are simultaneously introduced into the flow tube and reacted with a sample and the count rates measured. A ratio of the O_2^+ and NO^+ counts to the measured H_3O^+ counts is obtained. This ratio is then applied to the calculated collision rate for the H_3O^+

reaction to give the rate coefficients for the O_2^+ and NO^+ reactions. For some compounds, the relative method is the only feasible method of determining the reaction rate coefficient. This circumstance is especially true for VOC's with low volatility in reacting with the precursor, which limits the effectiveness of the absolute measurement approach.

Rate coefficients are generally transferable between different types of SIFT-MS instruments due to the technology's underlying chemistry - chemical kinetics. Because the rate coefficients are pressure and carrier gas independent, measurements performed on other instruments, using different flow tube pressures, or different carrier gases, should not alter the kinetic data obtained. However this point has yet to be fully validated experimentally.

2.4 QUANTIFICATION

SIFT-MS achieves quantification by reversing the technique used to determine reaction rate coefficients for a neutral gas reacting with an ionic species. As explained, reaction rate coefficients are found by measuring the change in precursor levels before and after the introduction of a reacting sample. SIFT-MS instead measures changes in the product ions formed from the reaction of the precursor and sample, and uses the previously determined reaction rate coefficient to convert the counts to a concentration. Therefore the more concentrated a product, the larger the proportion of precursor ions that are reacted to form product ions, and the greater the number of counts.

The reaction between an ion and a neutral species can then be described using first order kinetics as shown in Equation 2.9 (Syft, 2004). The count of product ion (*P*) in terms of the precursor ions is thus defined (Syft, 2004):

$$P = I_0 - I \qquad (2.11)$$

Substituting Equation 2.9 into Equation 2.11 gives (Syft, 2004):

$$P = I_0\left(1 - e^{-k[A]t}\right) \qquad (2.12)$$

Taking the ratio of P to I then gives (Syft, 2004):

$$\frac{P}{I} = \frac{I_0\left(1 - e^{-k[A]t}\right)}{I_o e^{-k[A]t}} \qquad (2.13)$$

For trace concentrations, where $k[A]t << 1$, taking the limit simplifies Equation 2.13 to the following form (Syft, 2004):

$$\frac{P}{I} = k[A]t \qquad (2.14)$$

The count of the product ion includes all possible products from a reaction. If more than one product is formed, it must be determined whether each product is primary, secondary, or higher order.

2.5 PRIMARY & SECONDARY PRODUCTS & BRANCHING RATIOS

Primary and secondary products, including their branching ratios are defined as reactions of the form:

$$A^+ + B \xrightarrow{k_1} C^+ \qquad 60\% \quad \text{Primary}$$

$$\xrightarrow{k_2} D^+ \qquad 40\% \quad \text{Primary}$$

$$C^+ + E \xrightarrow{k_3} F^+ \qquad \text{Secondary}$$

Primary product ions (C^+ & D^+) are products formed from the reaction of the original neutral ion in the sample compound (B) with the precursor (A^+). Secondary products (F^+) occur when a primary product ion goes on to react with another neutral species (E). Including secondary products makes the analysis more accurate, but can be ignored if the depletion of the primary products from secondary reactions is insignificant.

To find and classify the order of all products, the mass(es) of the product ion(s) and the proportions, termed branching ratios, in which they are formed must be known. The branching ratios therefore account for the contribution of each mass to the total products formed and sum to 1 if all products are monitored. When secondary products are included they are added to the total product count and divided by the same branching ratio as their original product ion. This same branching ratio value is used because they have come from the same pool of available primary products. The product masses and their branching ratios are found experimentally. Finally, it should also be noted that the precursor may also have multiple masses, which must be monitored.

2.6 MASS DISCRIMINATION AND DIFFERENTIAL DIFFUSION

As the precursor and product ions move along the flow tube they can diffuse to the walls of the flow tube. The diffusion of precursor ions and product ions is different and is termed differential diffusion (P Spanel & Smith, 2001).

Differential diffusion occurs because of differences in the mass of the product and precursor ions. The heavier product ions diffuse to the flow tube walls more slowly and are therefore more efficiently transported to the downstream mass spectrometer. The difference in transport efficiency results in falsely high count rates of heavier product ions (P Spanel & Smith, 2001). It is accounted for in the analysis using a coefficient for the product ions, D_e, which balances the differential diffusion and resulting differences in transport efficiencies of product and precursor ions.

Mass discrimination occurs when the quadrupole discriminates against heavier ions. It also includes the uneven sampling of different mass ions and the differential detection efficiencies. Mass discrimination is due to the settings used on the channeltron ion multiplier detector. It is accounted for using a coefficient, M_r, that gives the magnitude of discrimination for heavier product ions and lighter precursor ions (P Spanel & Smith, 2001).

In practice, the differential diffusion enhancement of heavier ions is counteracted by the mass discrimination of heavier ions (P Spanel & Smith, 2001). It has been found that for larger diameter flow tubes (>8 cm) the differential diffusion enhancement and discrimination of heavier product ions were small using high quality quadrapoles and therefore cancelled each other out (Davies, Spanel, & Smith, 1997; D Smith, Davies, & Spanel, 1999; P Spanel, Davies, & Smith, 1999). However, with the manufacture of

smaller SIFT-MS machines for commercial and clinical applications, correcting for the mass discrimination and differential diffusion is necessary to obtain accurate quantification.

The differential diffusion factor is combined with the mass discrimination factor to give an overall factor, D_f, (P Spanel & Smith, 2001).

$$D_f = \frac{M_r}{D_e} \qquad \textbf{(2 . 15)}$$

Where:

M_r = mass discrimination factor

D_e = differential diffusion factor

The combined factor has typical values ranging from 1 – 2.5 for m/z values of 15 – 200 respectively (P Spanel & Smith, 2001).

Rearranging Equation 2.14 and allowing multiple precursor masses and product masses, their branching ratios, the differing diffusion rates of the precursor and product ions and mass discrimination, the concentration of analyte in the flow tube is thus defined (Syft, 2004).

$$[A] = \frac{1}{t_r} \frac{\sum_{i=1}^{n} \frac{I_{si} D_{fi} + I_{\sec i} D_{fi}}{b_{ri}}}{\sum_{i=1}^{m} k_i I_{pi} D_{fi}} \qquad \textbf{(2 . 16)}$$

Where:

$[A]$ = analyte concentration (molecules cm^{-3})

t_r = reaction time (seconds)

I_{pi} = precursor ion counts for mass i (cps)

I_{si} = primary product counts for mass i (cps)

$I_{\sec i}$ = secondary product ion counts for mass i (cps)

k_i = rate coefficient (molecules cm^3 s^{-1}) for mass i

D_{fi} = combined differential diffusion & mass discrimination factor for mass i

b_{ri} = branching ratio for mass i

n = total number of product masses

m = total number of precursor masses

To convert [A] to units of ppb the following formula is used (Syft, 2004):

$$[A]_{ppb} = \frac{[A]T_g R\Phi_{Total}}{N_a \Phi_S p_g}\left(1\times10^{12}\right) \qquad (2.17)$$

Where:

R = gas constant (62.4 $LtorrK^{-1}mol^{-1}$)

N_a = Avogadros number ($6.022\times10^{23}\,mol^{-1}$)

$[A]$ = analyte concentration (molecules cm^{-3})

T_g = temperature of the gas in the flow tube (Kelvin, K)

Φ_{Total} = total flow in flow tube (torr Ls^{-1})

Φ_S = sample flow through capillary (torr Ls^{-1})

p_g = flow tube pressure (torr)

The factor 1×10^{12} arises from unit conversions of 1×10^{9} to change to ppb and $1000\times$ to account for difference in the units of R and $[A]$.

It can be seen from Equations 2.16 and 2.17 that the masses of precursors and products are used to calculate the concentration of the compound. Equations 2.16 and 2.17 hold when the concentration of the sample gas is very small ensuring the primary ion count rate is only slightly reduced by the reaction with a maximum of 10% reduction (D Smith & Spanel, 2005; D. Smith & Spanel, 1996). It is also possible that other compounds react with the same or a different precursor to produce products at the same mass unit. Therefore, it is important when a sample containing a selection of compounds is analysed that their product masses with the relevant precursor do not overlap. This situation is ensured by careful selection of precursor and product masses and omitting those which overlap. Once the products and reaction rate coefficients are identified for a compound and precursor their combination is commonly referred to as the kinetics.

2.7 REACTIONS OF PRECURSORS

This section analyses and presents several common precursor reactions used in SIFT-MS.

2.7.1 H3O+

The large majority of H_3O^+ precursor reactions with neutral ions occurs as an exothermic proton transfer (D Smith & Spanel, 2005). These exothermic proton reactions usually produce one or two product ions. For H_3O^+ this reaction is defined:

Exothermic Proton Transfer:

$$H_3O^+ + M \longrightarrow MH^+ + H_2O \qquad (2.18)$$

2.7.2 NO+

Compared to H_3O^+ reactions, NO^+ reactions also result in one or two product ions, but the reactions are more varied. Reactions include charge transfer, hydride ion transfer, hydroxide ion transfer, alkoxide ion transfer and ion-molecule association (D Smith & Spanel, 2005). It is also possible for more than one type of these reactions to occur in parallel. Of these reactions the majority are charge transfer and ion-molecule association. These reactions are defined:

Charge Transfer:

$$NO^+ + M \longrightarrow M^{+\bullet} + NO^\bullet \qquad (2.19)$$

Ion-Molecule:

$$NO^+ + M \longrightarrow NO^+M \qquad (2.20)$$

2.7.3 O2+

Reactions of O_2^+ include non-dissociative charge transfer or dissociative charge transfer (D Smith & Spanel, 2005). Reactions with O_2^+ are rapid, and like NO^+ and H_3O^+ result in the formation of one or two product ions. These reactions are defined:

Non-dissociative:

$$O_2^{+\bullet} + M \longrightarrow M^+ + O_2 \qquad (2.21)$$

Dissociative:

$$O_2^{+\bullet} + M \longrightarrow M^{+\bullet} + O_2 \qquad (2.22)$$

Overall, compounds can be analysed using multiple precursors, which should all give the same concentration. The agreement of the concentration from each precursor is an excellent way to check that the reported concentration values are correct.

2.8 EFFECT OF WATER ON QUANTIFICATION CALCULATIONS

All air samples are humid, with a relative humidity of around 1-2%, while breath samples are very humid, with a relative humidity of around 6%. Therefore, if SIFT-MS is used for quantification of compounds in breath and air, it must also account for the effect of water vapour (D Smith & Spanel, 2005; P Spanel & Smith, 2000). Water vapour affects the analysis through the formation of hydrated ions of the precursor and product ions that can continue to react with the sample (P Spanel & Smith, 2000). However, the influence of water molecules is dependent on the precursor ion chosen. The most affected precursor ion is H_3O^+, due to the formed hydrated hydronium ions $H_3O.(H_2O)_{1,2,3}$ that result in a typical error of 15 % for samples at breath humidity levels (D Smith & Spanel, 2005; P Spanel & Smith, 2000). For NO^+ (Ferguson, Fehsenfeld, & Schmeltekopf, 1969; IkezoeY, S, M, & Viggiano, 1987; P Spanel & Smith, 2000) and O_2^+ (D Smith & Spanel, 2001; P Spanel & Smith, 2000) the ion reactions involving hydrated hydromium ions ($NO^+.H_2O$ and $O_2^+.H_2O$) is a slow association reaction and therefore it is not necessary to include. This is an assumption

based on the small contributions to the overall products that these association reactions contribute.

A new calculation method for compound concentration [*A*] in humid samples using H_3O^+ precursor was proposed by Spanel and Smith (2000). The new equation reduces the error of 15% to 2% for quantification of compounds from samples at breath humidity levels. This equation alters the reaction rate coefficients, k_i, in Equation 2.16 which can be written using the H_3O^+ precursor as:

$$[A] = \frac{1}{t_r} \frac{\sum_{i=1}^{n} \frac{I_{si} D_{fi} + I_{\sec i} D_{fi}}{b_{ri}}}{[I_{p19}] k_{19} D_{f19} + [I_{p37}] \frac{k_{19} D_{f19} + k_{37} D_{f37}}{2} + [I_{p55}] \frac{k_{19} D_{f19} + k_{55} D_{f55}}{2} + [I_{p73}] \frac{k_{19} D_{f19} + k_{73} D_{f73}}{2}} \quad \textbf{(2 . 23)}$$

Where:

$[A]$	=	analyte concentration (molecules cm^{-3})
t_r	=	reaction time (seconds)
$I_{p19,37,55,73}$	=	precursor ion counts for masses 19,37,55,73 (cps)
I_{si}	=	primary product counts for mass *i* (cps)
$I_{\sec i}$	=	secondary product ion counts for mass *i* (cps)
$k_{19,37,55,73}$	=	rate coefficient (molecules cm^3 s^{-1}) for masses 19,37,55,73
D_{fi}	=	combined differential diffusion and mass discrimination factor for mass *i*
$D_{f19,37,55,73}$	=	combined differential diffusion and mass discrimination factor for masses 19,37,55,73

b_{ri} = branching ratio for mass *i*

n = total number of product masses

2.9 MEASUREMENT OF HUMIDITY

Humidity is the concentration of water vapor in a sample. The concentration is typically expressed as an absolute humidity. Absolute humidity is expressed as the number of grams of water vapor per cubic meter of air. It is possible to express the absolute humidity as a percentage, meaning that an absolute humidity value of 6% indicates that 6% of the sample is water vapor by mass.

It is possible to measure the absolute humidity of a sample with SIFT-MS using the H_3O^+ precursor. This measurement is done using the relative amounts of the H_3O^+ ions and H_3O^+. $(H_2O)_{1,2,3}$ hydrate ions in the carrier gas and can be done at the same time as monitoring a compound's concentration (D Smith & Spanel, 2001). The formation of the hydrate ions occurs according to sequential three body association reactions. Using helium as the stabilising third body the reaction is thus defined:

$$H_3O^+ + nH_2O + He \longrightarrow H_3O^+.(H_2O)_n + He \quad n = 1,2,3 \qquad \textbf{(2.24)}$$

Therefore the H_3O^+. $(H_2O)_{1,2,3}$ form sequentially down the flow tube.

A method for the calculation of absolute humidity using SIFT-MS was given by Spanel and Smith (2001). The number density of water molecules in the carrier gas is given by:

$$[H_2O] = \frac{1}{t_r k_{2eff}} \ln \frac{\{H_3O^+\}D_{f1} + \{H_3O^+.H_2O\}D_{f2} + \{H_3O^+.(H_2O)_2\}D_{f3} + \{H_3O^+.(H_2O)_3\}D_{f4}}{\{H_3O^+\}D_{f1}} \quad \textbf{(2 . 25)}$$

Where:

$\{H_2O\}$ = number density of water molecules (molecules cm^{-3})

t_r = reaction time (seconds)

$\{H_3O^+.(H_2O)_{0,1,2,3}\}$ = product or precursor ion counts (cps)

k_{2eff} = effective two-body rate coefficient(molecules cm^3 s^{-1})

D_{fi} = correction factor for mass discrimination and differential diffusion for the relevant mass

1 = $\{H_3O^+\}$; 2 = $\{H_3O^+.H_2O\}$; 3 = $\{H_3O^+.(H_2O)_2\}$;

4 = $\{H_3O^+.(H_2O)_3\}$

The absolute humidity, H_{AH}**,** can therefore be found from the $H_3O^+.(H_2O)_{0,1,2,3}$ ion count rates (D Smith & Spanel, 2001):

$$H_{AH} = \frac{[H_2O]}{3.54\times10^{16}} \frac{T_g \Phi_{Total}}{T_{ref} \Phi_S p_g} \quad \textbf{(2 . 26)}$$

Where:

T_g = temperature of the gas in the flow tube (Kelvin, K)

p_g = flow tube pressure (torr)

T_{ref} = reference temperature for rate coefficients (273 K)

H_{AH} = relative humidity (%)

Φ_{Total} = total flow in flow tube (torr Ls^{-1})

Φ_S = sample flow through capillary (torr Ls^{-1})

$[H_2O]$ = number density of water molecules (molecules cm^{-3})

2.10 MACHINE SPECIFICATIONS

Like any sensor, it is important that the behaviour of SIFT-MS is known and that it is an accurate and absolute analytical technique. The behaviour can be described by a number of factors.

2.10.1 ACCURACY, PRECISION & REPEATABILITY

Accuracy describes the difference between the value found and the value that is accepted as true, and is linked to the precision. Precision describes the scatter in values obtained from measurements of the same homogeneous sample under identical conditions. Repeatability is how the precision varies over time, between tests or different days.

Studies have been done to investigate the precision and accuracy for the analysis of trace gases in dry air using SIFT-MS. Several organic vapors have been tested including ethanol, benzene, toluene, xylene, acetone, 2-butanone, 1-methoxy-2-propanol, and trichloroethylene, over a range of concentrations using prepared standard atmospheres (P Spanel, Cocker, Rajan, Smith, & 1997) and permeation tubes (D Smith et al., 1998). These studies reported an accuracy of better than 10% over a range of 10 parts per billion (ppb) to 20 parts per million (ppm) (P Spanel, Cocker, Rajan, Smith, & 1997; D Smith et al., 1998). No study on the repeatability of SIFT-MS has been reported to date.

2.10.2 SPECIFICITY

The specificity is the ability to clearly monitor the analyte in the presence of other components. When selecting compounds to monitor the masses must not overlap, otherwise recordings will involve counts for both masses. The specificity is also affected by the presence of water in the sample as discussed earlier (P Spanel & Smith, 2000;D Smith & Spanel, 2005).

2.10.3 LIMIT OF DETECTION (LOD) & RANGE

The LOD describes the lowest amount of analyte that can be quantitatively determined with suitable precision and accuracy. The detection limits of SIFT-MS using typical machine settings and reaction rate coefficients is 1 ppb for an integration time of 1 s at a product ion count rate of 1 c/s (D. Smith & Spanel, 1996). The highest concentration is limited by the assumption in Equation 2.16 and 2.17 that the depletion of the precursor count rate does not exceed approximately 10% of their initial values (D Smith & Spanel, 2005). The range is therefore largely dependent on the sample flow rate into the flow tube and available precursor.

2.11 APPLICATIONS OF SIFT-MS

SIFT-MS has many potential applications from environmental science to medicine to security and other fields. Figure 2.4 shows many of these potential applications schematically by broader field.

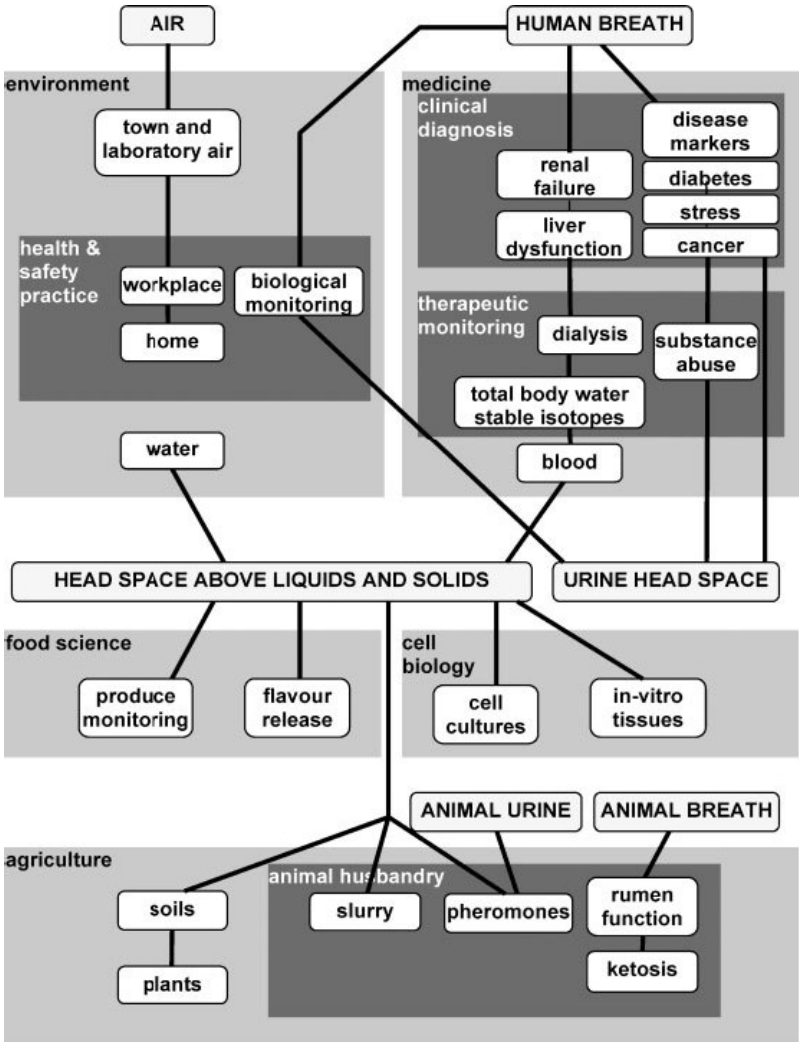

Figure 2.4: Applications of SIFT-MS (D Smith & Spanel, 2005)

2.12 SUMMARY

SIFT-MS is a mass spectrometric method that allows simultaneous quantitative analyses of several trace gases in air and exhaled breath down to ppb levels in real time. SIFT-MS uses selected positive precursor ions (H_3O^+, O_2^+, NO^+) to react with VOC's (1-300amu) in samples to form particular product ions which are detected and used to indicate the VOC's present in the sample and their concentration. Quantification is achieved by measuring the count ratio of precursor and products

formed and knowing the rate at which these reactions occur. The product ions include all possible products from a reaction including their relevant branching ratios. The reaction rate coefficient describes the speed at which reactions occur between precursor and product ions. There is also an allowance made for mass discrimination and differential diffusion.

Water vapour affects the specificity of SIFT-MS, depending on the precursor ion used. The most affected precursor ion is H_3O^+, while NO^+ and $O2^+$ are least affected. No study on the repeatability of SIFT-MS has been reported to date.

SIFT-MS is an emerging technology which has many potential applications from environmental science to medicine to security and other fields. It is this ability to monitor VOC's in whole air samples in real time that provides a unique opportunity for breath test diagnosis.

This chapter has described the workings of the SIFT-MS instrumentation while the next chapter will discuss potential mediums to store breath and methods to remotely collect breath samples.

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3 REMOTE SAMPLE COLLECTION METHODS

Remote collection of breath samples is necessary for many reasons. The current SIFT-MS machine at Christchurch Hospital (LDI2) and even the most recent Voice-100 SIFT-MS machines are not practically portable, especially in a hospital environment. The portability of the LDI2 and Voice-100 SIFT-MS instruments is hindered by their physical size and resource requirements. They are also costly which means having many machines within any one establishment is not financially ideal. The noise level generated by SIFT-MS may also be inappropriate in a clinical setting. Therefore, bringing a SIFT-MS in currently available configurations to numerous patients within a hospital environment is impractical.

It is not always possible to bring the patient to a SIFT-MS instrument for testing. The patients could be elderly or newborn, healthy or sick, ambulatory bodied or immobile, even unconscious. It is therefore currently impracticable and unfeasible to bring SIFT-MS to patients or patients to a SIFT-MS instrument. The best action is therefore to collect patient samples remotely and transport them to SIFT-MS instrument for testing. This approach will optimise the throughput, and thus cost efficiency of the machine.

3.1 REMOTE SAMPLING REQUIREMENTS

Remote collection of breath samples for disease diagnosis by SIFT-MS requires certain criteria be met by the collection method. The method ideally should (Wilson & Monster, 1999; Raymer, Thomas, Cooper, Whitaker, & Pellizzari, 1990 ; Phillips, 1997):

- enable quantitative results from collected samples
- maintain sample integrity
- not cross-contaminate samples through the collection and removal methods
- collect a wide range of compounds
- involve simple collection and removal methods
- be compatible with humidity
- be compatible with analysis with SIFT-MS
- be safe and hygienic
- require minimal effort to give a sample
- enable remote collection
- be inexpensive
- enable rapid collection and recycle time
- facilitate alveolar breath collection

These criteria form the design specification for the collection method and are useful in evaluating different collection methods. Hence, it is possible to choose the best collection method for analysis by SIFT-MS.

The current methods used to remotely collect breath fall within two main categories, adsorbent resins and containers. Adsorbent resins and containers enable quantitative results, facilitate alveolar collection, are safe and hygienic and collect a wide range of compounds. However, they differ when meeting the other requirements of a remote breath collection method. Containers are simply volumes used to store a sample within. Containers are usually made from a plastic material, but other rigid containers, such as stainless steel canisters, have been used in the past (Raymer, Thomas, Cooper, Whitaker, & Pellizzari, 1990 ; Wilson & Monster, 1999). However, because SIFT-MS relies on a constant sampling flowrate created by the pressure differential between the sampling capillary and flowtube, rigid containers cause problems as their pressure alters over time as they are evacuated.

Adsorbent resins are substances that trap and hold compounds from samples and then release them for analysis through a process known as thermal desorption. Thermal desorption involves heating the resin to a high temperature and passing an inert gas over it to remove the collected compounds. The trapped analytes become pre-concentrated because they are collected from a large volume at low temperatures and then desorbed using a smaller flushing volume at high temperatures into a detector. Knowing the original sample and flushing volumes then allows calculation of the original sample concentration. The compounds are then analysed using a detection technique such as GC-MS or SIFT-MS. The effectiveness of pre-concentration is dependent on the relationship between the adsorbed and desorbed volume. For pre-concentration the adsorbed volume needs to be greater than the desorbed volume. Many commercially available adsorbents can be used to collect breath samples

remotely. Due to current desorption facilities available during this study, Tenax™ TA and Solid Phase Micro-Extraction (SPME) were the primary materials considered.

3.2 COLLECTION METHODS USED IN CURRENT DEVICES

There are a number of devices available which collect breath. These products used a range of collection methods for storing the breath samples and a range of compounds and concentrations, as shown in Table 3.1. The majority of the products collect breath onto adsorbent traps and are analysed using GC-MS after thermal desorption. Using GC-MS largely influences the use of adsorbents for collection, because there is more sample preparation required for analysing whole air samples compared to adsorbents with GC-MS. Analysing adsorbents is also automated, requiring little user input. SIFT-MS is more capable of dealing with whole air samples than GC-MS and therefore can easily deal with sampling from containers.

Ref	Collection Method	Collection Time	Collected Compounds	Cocentration
1	Direct onto adsorbent	5 mins	ethanol, pentane, isoprene	Not given
2	Direct into stainless steel canisters	2 mins	pentane, benzene, chloroform	0.120-560 ppm
3	Glass Syringe	4 mins	hydrogen	Not given
4	Tedlar bag then transfer to adsorbant	< 1 min	toluene, xylene	0.160-43 ppm
5	Direct onto adsorbant	5 mins	isoprene, accetone, pentane	30-60 ppm

Table 3.1: Collection methods used by current devices used to collect remote samples of breath (1)Phillips, 1997 (2)Raymer, Thomas, Cooper, Whitaker, & Pellizzari, 1990 (3)Yeung et al., 1991 (4)Dyne, Cocker, & Wilson, 1997 (5)Schubert, Spittler, Braun, Geiger, & Guttmann, 2001

3.3 SOLID PHASE MICRO-EXTRACTION (SPME)

SPME involves stainless steel fibres that are coated with an analyte adsorbing liquid-phase material such as polydimethylsiloxane (PDMS), polyacrylate (PA) or carbowax (Grote & Pawliszyn, 1997). Choice of the right fibre with appropriate polarity and stereoselectivity depends on the physicochemical properties of the analytes

(Pawliszyn, 1997). Different SPME fibre coatings use equilibrium, non-equilibrium or both processes to concentrate and collect samples (Prince, 2004).

The amount of analyte that dissolves into the liquid phase is governed by the concentration of analyte in the sample and the affinity of the analyte for the liquid phase. This is an equilibration process. The equilibrium constant is given by (Prince, 2004):

$$K_{fg} = \frac{c_f}{c_g} \qquad (3.1)$$

Where:

c_f = analyte equilibrium concentration in the fibre liquid phase

c_g = analyte equilibrium concentration in the gas phase

K_{fg} = equilibrium constant

Where K_{fg} is specific to a compound and fibre coating and indicates how much more concentrated the analyte is in the fibre coating compared to the gas sample. Its value typically ranges between 10^2-10^5.

In addition to the liquid phase, some SPME fibres have solid phase particles suspended in the liquid phase, for example carboxen (CAR) or divinylbenzene. The solid phase adsorbs analyte to a level that is governed by the total surface area of the solid phase. This is not an equilibrium process.

To obtain quantitative results from SPME, standards containing amounts of the pure analytes are made at a range of concentrations (Grote & Pawliszyn, 1997;Pawliszyn,

1997). Each standard at its relevant concentration is adsorbed onto the SPME. The SPME is then desorbed and the concentration of desorbed analyte found using an analysis technique. Repeating the adsorption and desorption for a range of sample concentrations gives a set of calibration curves, which link the concentration of analyte absorbed onto the SPME to the concentration of the original sample.

3.3.1 METHOD OF USE & PHYSICAL DESCRIPTION

SPME is commercially available as single fibres housed in a protective covering as shown in Figure 3.1. The fibre diameter and volume is dependent on the type of coating used and amount of analyte that is to be adsorbed. Common fibre liquid phase diameters used for breath analysis range between 7-100 μm and have a coating volume of 0.026 – 0.612 mm^3 respectively (Grote & Pawliszyn, 1997).

To collect a sample, the SPME fibre is exposed to the sample (Grote & Pawliszyn, 1997;Pawliszyn, 1997). No supplementary equipment is required. To analyse the compounds trapped on the SPME it is thermally desorbed and purged with an inert carrier gas such as helium. The mixture is then sent to the detector (SIFT-MS) for analysis.

The SPME may then be reused after it has been thermally desorbed to release all previously stored compounds. Thermal desorption of liquid phase SPME coatings takes approximately 2-3 mins at 200-280°C. Solid phase coatings, which hold their trapped compounds more tightly, require higher temperatures (340°C) and longer times.

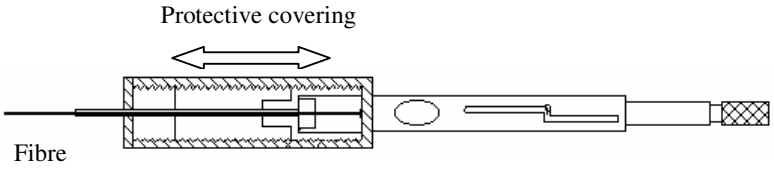

Figure 3.1: Commercially available device containing the SPME fibre and its protective cover

3.3.2 SUITABILITY OF SPME FOR BREATH ANALYSIS USING SIFT-MS

A significant problem for using SPME for breath analysis with SIFT-MS is the small volume of adsorbent material on the SPME fibres in relation to the sample volume requirements of SIFT-MS. An ideal sample time for SIFT-MS would last 10-20 sec, during which time the capillary would have drawn approximately 35mL into the flow tube. Therefore, any purge volume used during thermal desorption of the SPME would need to be at least 35mL.

However, this volume is 5.7×10^4 times larger than the largest liquid phase SPME coating volume available. Thus, any concentration factor, K_{fg}, of the SPME needs to be greater than 5.7×10^4, which lies at the upper limit of the liquid phase SPME coatings. Other studies using SPME for breath analysis using GC-MS have found the small adsorbent volume limits the method to compounds with relatively high concentrations in human breath greater than 100 ppb (Grote & Pawliszyn, 1997). Therefore, liquid phase coatings do not have a high enough adsorbent volume to measure concentrations at breath levels using SIFT-MS, or to increase the sensitivity of SIFT-MS through pre-concentration.

Solid phase adsorbent coatings have higher adsorption capabilities than the liquid phase coating, and can therefore be used to overcome the issues associated with small

adsorbent volumes. However, solid phase adsorbent coatings adsorb according to a non-equilibrium process and are therefore problematic to calibrate for quantitative results.

It is assumed when calibrating SPME that the calibration sample, which often contains only one analyte, adsorbs onto the SPME in a similar fashion to the actual sample, which contains a mixture of analytes. This assumption does not work well for a heterogeneous sample such as breath. It is possible to use calibration samples that are similar to breath, but this is complicated due to difficulties with making standards that accurately reflect breath. Calibration works well for the liquid phase SPME coatings, which adsorbs according to an equilibrium process. Calibration for solid phase SPME coatings is a non-equilibrium process and experiments must therefore be completed into the linearity of adsorption and saturation level of these fibre coatings before clinically relevant quantitative results can be obtained.

SPME is problematic for large scale screening and testing. Calibration is not universal and must be carried out for each fibre. Separate calibration is required due to manufacturing differences that result in varying amounts of adsorbent on each SPME fibre. In addition, the solid phase coatings such as Carboxen are coated by hand, while the liquid phase fibres, such as PDMS, are machine coated, both of which can lead to additional variabilities. A particular advantage of SPME is that samples can be stored for up to 8h without significant loss, and the fibres do not adsorb H_2O or CO_2 (Grote & Pawliszyn, 1997). Overall, a SPME summary includes:

Advantages:

- does not adsorb H_2O or CO_2
- very convenient sample collection with minimal associated equipment
- samples can be stored for up to 8h
- reusable

Disadvantages:

- Adsorbent volume on liquid phase coatings is too small to measure breath levels for analysis with SIFT-MS or increase the sensitivity of SIFT-MS
- quantification is difficult for complex mixtures at breath levels (ppb)
    - breath is a heterogeneous mixture and making up standards to accurately reflect breath is difficult especially at levels bellow ppm
    - calibration is required separately for each SPME fibre
    - solid phase coatings adsorb in a non-equilibrium process

3.4 TENAX™ TA

Tenax™ TA is a porous polymer based on 2,6-diphenylene oxide (Scientific Instrument Services, 2005c). Tenax™ TA is a granular inert compound used for trapping volatile and semi-volatile analytes from liquid or solid samples. To trap compounds, the sample is passed through a bed of Tenax™ TA, which acts like a sieve. Unlike SPME, Tenax™ TA is capable of holding much greater amounts of compounds from samples. It therefore adsorbs enough compound to ensure the 35mL

sample volume required by SIFT-MS has a higher concentration level than 35mL of the original sample.

Selecting Tenax™ TA for the trapping of analytes involves consideration of a number of physical parameters and characteristics of the analyte of interest and adsorbent resin (Manura, 1995):

- breakthrough volumes (see later) for analytes at sample collection temperature
- water in sample and affinity of Tenax™ TA for water
- sampling flowrate and sample volume which gives the collection time
- range of organic analytes to be sampled
- concentration of analytes in gas sample
- detection limits of detector (SIFT-MS)
- amount of Tenax™ TA to be used

Using Tenax™ TA to quantify compounds within samples involves assumptions about the movement of the sample through the Tenax™ TA. It is assumed that a sample moves through the Tenax™ TA as a packet whose concentration forms a normal type distribution with tube position, as shown in Figure 3.2.

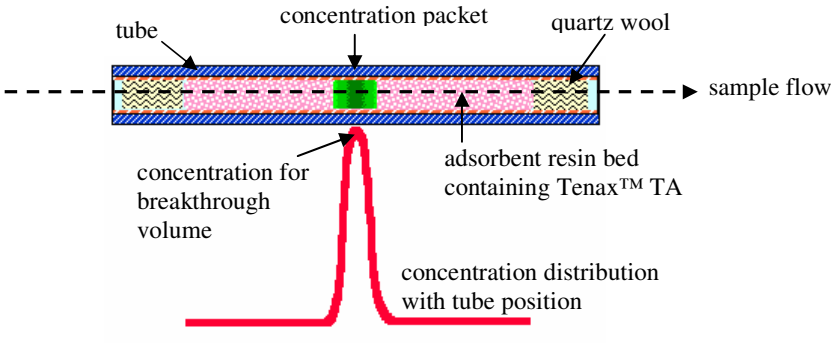

Figure 3.2: Tenax™ TA assumed sample parameters (Manura, 1995)

The breakthrough volume is related to the volume of sample that causes an analyte to migrate through the adsorbent resin bed. When collecting samples, the breakthrough volume cannot be exceeded to trap all the analyte. It is important to trap all the analyte so that the amount of analyte trapped in the Tenax™ TA is representative of the amount within the volume sampled. Trapping all the analyte from the volume sampled is therefore the only way to obtain quantitative, clinically relevant results.

Breakthrough volume values have been published by Scientific Instrument Services for a range of compounds for dry samples at a range of temperatures (Scientific Instrument Services, 2005b) as shown in Table 3.2. However, not all compounds commonly found in breath, such as isoprene and ammonia, have tabulated data. It should also be noted that several parameters distort or alter the breakthrough volumes published. These include the presence of water, sampling flow rate, amount of Tenax™ TA and competition for active trapping sites.

Compound	20°C	40°C
Ethanol	1.800	0.481
Pentane	5.000	1.100
Acetone	6.000	1.400
Isoprene	not available	not available
Ammonia	not available	not available

Table 3.2: Breakthrough volumes for Tenax™ TA in Lgram^{-1} for different temperatures

As discussed, breakthrough volumes are calculated by assuming a packet of the sample moves through the Tenax™ TA. However, this dynamic is not representative for adsorbing onto the Tenax™ TA over time. When the sample is continuously added to the adsorbent resin bed, there can be competition for the active sites (Comes,

Gonzalez-Flesca, Menard, & Grimalt, 1993;Harper, 1993). The competition for active sites reduces the breakthrough that occurs.

Tenax™ TA has a low affinity for water and is therefore useful for collecting volatile organic compounds from high moisture content samples, such as breath. The effect of water in the samples is negligible as long as the water is kept in the gas phase (Harper, 1993; Pankow, 1988; Seshadri & Bozelli, 1983). Hence Tenax™ TA may be appropriate for clinical breath testing applications.

The velocity of the carrier gas can also affect the breakthrough volume data during sampling (Seshadri & Bozelli, 1983; Riba, Clement, Haziza, & Torres, 1991). If the carrier gas velocity is too high, the analytes move through the adsorbent at rates that do not permit them to interact with the pores of the adsorbent resin (Riba, Clement, Haziza, & Torres, 1991). The maximum sampling flow rate is mainly dependent on the Tenax™ TA tube diameter. The maximum sampling flow rate through a 3.0 mm diameter tube is 150 ml/min, and 50 ml/min for a 4.0 mm tube (Manura, 1995). Hence, peak flow rates must also be controlled for any given application.

The amount of Tenax™ TA used depends on both the analyte concentration in the original sample and the detection limit of the detector (SIFT-MS). As discussed, Tenax™ TA effectively concentrates the analyte by collecting it from a large volume at low temperatures and then desorbing using a smaller flushing volume at high temperatures. Pre-concentration is necessary to analyse analytes whose original sample concentration is outside the detection limits of the detector (SIFT-MS). Once the required pre-concentration is known, the volume of the sample can be found and

the amount of Tenax™ TA calculated so that the breakthrough volume is not exceeded.

3.4.1 METHOD OF USE AND PHYSICAL DESCRIPTION

Tenax™ TA is purchased as a granular power and inserted into glass tubes with quartz wool at either end to retain the Tenax™ TA. The diameter of the glass tubes are largely controlled by the allowable size for the thermal desorption machine. Typical diameters range between 3 – 4 mm (Scientific Instrument Services, 2005a).

To collect a sample, a constant flowrate sampling pump is attached to one end of the Tenax™ TA tube. The sample is then pulled through the Tenax™ TA tube, as shown in Figure 3.3. The flowrate is measured along with the sampling time to determine the sampled volume.

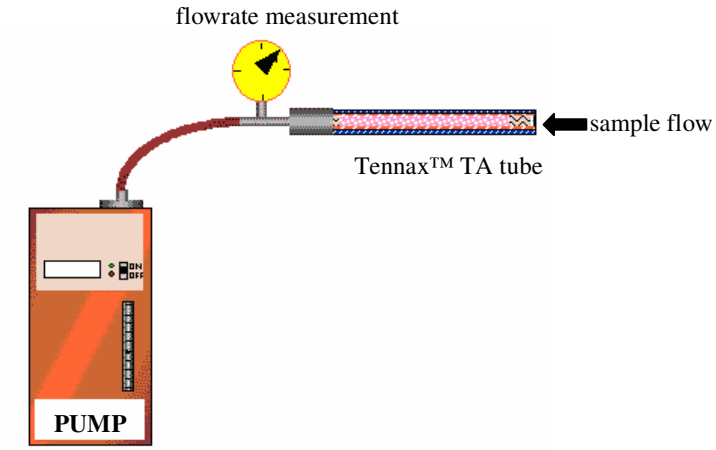

Figure 3.3: Tenax™ TA sampling method (Manura, 1995)

Once the sample is collected, the Tenax™ TA is thermally desorbed at temperatures between 150-300°C, and purged with an inert carrier gas such as Helium. This mixture is then sent to the detector (SIFT-MS) for analysis. Tenax™ TA may be re-used only after it has been thermally desorbed to release all previously stored

compounds. Thermal desorption takes approximately 5-10mins. Finally, Tenax™ TA will naturally adsorb a small amount of compound from the atmosphere during storage, but adsorption is minimised by using Teflon caps placed over the ends of the tube containing the Tenax™ TA.

3.4.2 SUITABILITY OF TENAX™ FOR BREATH ANALYSIS

To apply Tenax™ for breath collection, each analyte to be trapped must be evaluated as to the feasibility of using techniques of purge and trap thermal desorption. Analyte recoveries using Tenax™ TA vary widely due to purging efficiency, purge apparatus design, choice of adsorbent, purge temperature and many other factors (Manura, 1995). Each individual analyte presents unique problems that must be overcome to achieve accurate and precise quantification. Analytical strategies must be formulated on an individual basis and are not always universally applicable. Intensive methods development and validation studies are therefore required for each individual analyte combination to guarantee accuracy and precision in the measurements.

Sampling with Tenax™ TA while patients are present can take considerable time, depending on the tube diameter and sample volume. For example, a 1L breath sample would take up to 7 mins using a 3 mm diameter tube and the device made by Phillips (1997) takes 5 mins. To ensure sampling takes minimal time for patients, a sample is often collected into a container and later passed through the Tenax™ TA (Wilson & Monster, 1999;Dynea, Cockerb, & Wilson, 1997; Baumbach, Vautz, Ruzsanyi, & Freitag, 2005). Having to store the sample within a container prior to sampling is even more time intensive and involves numerous extra steps that may lead to loss of analytes.

The storage time of compounds on Tenax™ TA varies depending on the storage conditions and trapped compounds. Storage times of up to 14 - 25 months (Health and Safety Laboratory, 1997) and 1-4 weeks (Center for Environmental Research Information Office of Research and Development, 1999) have been reported. Overall the use of Tenax™ TA can be summarised as follows:

Advantages:

- quantification is possible as long as breakthrough volume is not exceeded
- pre-concentration enables detection of analytes whose sample concentration is outside SIFT-MS detection limits
- reusable

Disadvantages:

- sampling apparatus is complex involving accurate heating and flow measurement
- direct sampling takes considerable time, and pre-sampling into a container adds complexity and increased potential for analyte loss
- collection method must be formulated on an individual analyte basis and are not universally applicable
- intensive methods development and validation studies are required
- breakthrough volume is affected by many factors which are difficult to control

3.5 TEDLAR™ BAGS

Tedlar™ bags are gas sample bags made from polyvinyl-fluoride (PVF) whose commercial name is Tedlar™. PVF is a fluoroplastic that has excellent chemical and temperature resistance, good aging properties, remains tough and flexible over a broad temperature range, and contains no plasticizers (Dupont, 1995a; Dupont, 1995b). Hence, it is a highly viable material for breath testing.

Tedlar™ film is manufactured in a wet solvent process and continues to emit organic compounds after manufacturing. New commercial Tedlar™ bags have measurable concentrations of phenol N,N-dimethylacetamide (Kelly, 1985; Chase, 2001; Koziel et al., 2004; Parker, Rhoades, Koziel, & Spinhirne, 2003). The presence of phenol N,N-dimethylacetamide was tested by mass scans of 3L brand-new Tedlar™ bags filled with nitrogen. The scans indicated that Phenol and N,N-dimethylacetamide were present at levels of 200-500 ppb for Phenol and 500-700 ppb for N,N-dimethylacetamide. The product masses from reactions with precursors are shown in Table 3.3.

Precursor	Phenol Mass (amu)	N,N-dimethylacetamide Mass (amu)
H_3O^+	95	88
O_2^+	94	87
NO^+	94	87, 117

Table 3.3: Product masses monitored from reaction of precursors with Phenol and N,N-dimethylacetamide

3.5.1 METHOD OF USE AND PHYSICAL DESCRIPTION

Tedlar™ bags, as shown in Figure 3.4, come in standard 0.5L, 1L and 3L sizes, but can be custom made to any size. They can be transparent, or black for photo-sensitve compounds, and have a range of options for the side port material including Teflon, stainless steel and Polypropylene (SKC Inc). To obtain a sample it is simply collected into the bag through the side port. Common flowrates for collection are around 3L/min (Environmental Response Team, 2003).

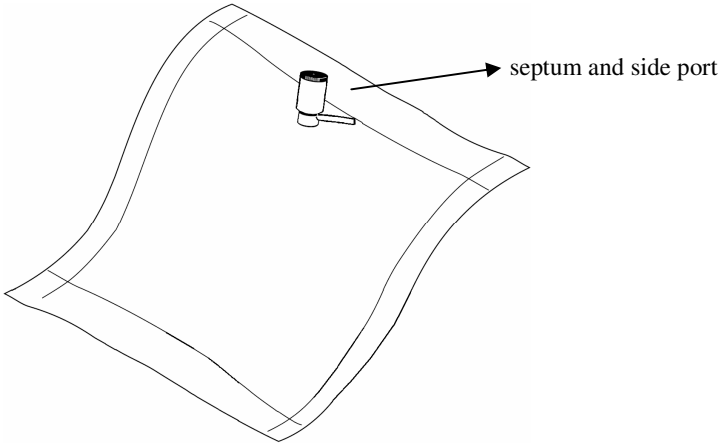

Figure 3.4: Tedlar™ bag in a deflated state

3.5.2 SUITABILITY OF TEDLAR™ BAGS FOR BREATH ANALYSIS

The use of polymer bags for the collection of gas samples is well established and has become one of the most common means of breath sample collection (Schuette, 1967;Smith & Pierce, 1970). The agreement in concentration levels from samples taken from a Tedlar™ bag and three direct breath measurements as shown in Figure 3.5, indicates the suitability of the method.

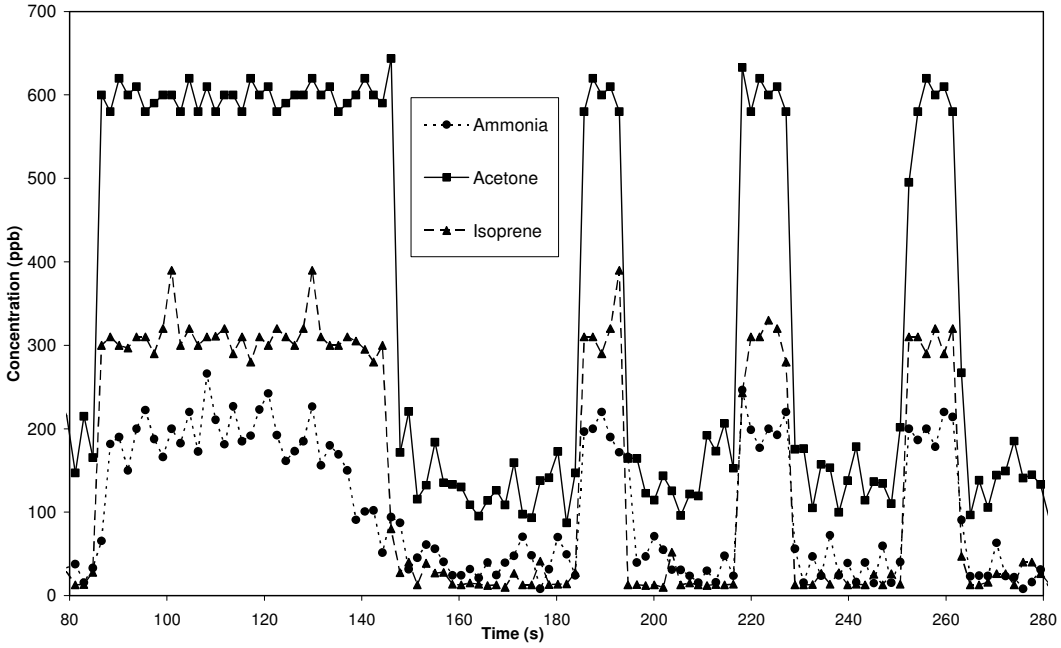

Figure 3.5: SIM Scan analysis of breath firstly from a Tedalr bag followed by three subsequent breaths directly into the SIFT-MS instrument

Due to the chemical structure of Tedlar™, highly polar compounds adhere to the inner surface of the bag and low molecular weight compounds may permeate the bag (Environmental Response Team, 2003; McGarvey & Shorten, 2000). The more reactive the compound, the less time it may be kept in the bag before the sample results begin to be compromised. Storage times should be less than 4 hours, but is largely dependent on the compound, its concentration, other compounds present, bag geometry and storage conditions such as temperature (Posner & Woodfin, 1986; McGarvey & Shorten, 2000).

It is possible to reuse Tedlar™ bags for some applications. It is not recommended to reuse bags after sampling compounds found at ppb concentrations, reactive compounds, or compounds that are known to adhere to the surface of the bag (Keika Ventures, 2003). Prior to reuse, the bags must be evacuated and thoroughly cleaned

and flushed after each use with purified nitrogen (McGarvey & Shorten, 2000). The design features of Tedlar™ bags may be summarised as follows:

Advantages:

- light, non-breakable and inexpensive
- simple collection methods
- quantitative results are possible

Disadvantages:

- bag reuse for breath levels (ppb) may not be possible
- integrity of samples gives low storage time
    - highly polar compounds adhere to the inner surface of the bag
    - low molecular weight compounds may permeate the bag
- out gassing of phenol N,N-dimethylacetamide causing sample contamination

3.6 SUMMARY

Because it is not always feasible to bring patients to the SIFT-MS instrument or a SIFT-MS instrument to patients, it is best to collect patient samples remotely and transport them to the machine for testing. Thus, it is necessary to choose a collection method for breath samples. There are a number of requirements for any remote sampling method. Current methods used to remotely collect breath include adsorbent resins and containers. Tedlar™ bags were the considered container while Tenax™ TA and Solid Phase Micro-Extraction (SPME) were considered adsorbent resins.

Although SPME facilitates fast and easy sampling, quantification is difficult due to the calibration method and heterogeneous nature of breath samples. Obtaining quantitative results from Tenax™ TA involves intensive method development and validation studies for each individual analyte combination. Tedlar™ bags are easy to use and enable quantitative measurements using SIFT-MS. Because SPME and Tenax™ TA both involve complex methods for quantification, including the associated equipment, it was more expedient to use Tedlar™ bags in this study.

Overall, when using remote sample collection methods for disease diagnosis using breath tests, it is important that the integrity of the sampling method is well understood. It was therefore necessary to develop a methodology for establishing the integrity for storing breath in Tedlar™ bags and to apply the methodology developed to determine its integrity. This task will be presented in chapter 4.

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4 FACTORS AFFECTING SAMPLE INTEGRITY

The integrity of a sampling method describes its ability to keep a sample in its original form. The integrity of a sample describes how close it is to its original form. There are many aspects of a sample which can be used to describe its integrity. Two important aspects of the sample integrity are changes in the compound concentration and humidity with time. Therefore, when ever sample integrity is referred to within this thesis, it is describing the changes in compound concentration or humidity with time.

When using remote sample collection methods for disease diagnosis using breath tests it is important that the sampling method has great integrity, or that changes in the sample integrity are well understood. It is also important to have in place a methodology for evaluating the integrity of the sampling method and determine the accuracy, robustness and repeatability of that integrity.

The remote sampling method chosen was Tedlar™ bags and it was therefore necessary to:

- develop a methodology for establishing its integrity for storing breath
- apply the developed methodology to determine its integrity for storing breath

As a first step in developing a methodology to determine the sample integrity, the purpose or aim of the methodology must be established. This aim must include central factors important in establishing the sample integrity of the storage method, in this case Tedlar™ bags.

4.1 TESTING FACTORS

There are many factors that would affect the integrity of breath samples stored in Tedlar™ Bags. These factors include (Schuette, 1967; Posner & Woodfin, 1986):

- surface area to volume ratio of the container (storage size)
- duration of storage
- concentration of the stored compounds
- humidity of the sample
- storage temperature
- physical and chemical properties of the plastics including their adsorption and diffusion characteristics
- pressure in the bag
- emission of compounds from the bag material
- presence of reactive compounds in the sample
- inter-bag variation
- characteristics of the compounds

Each factor could be tested to identify the extent and nature of their effect. However, the development of a methodology for establishing the sample integrity could be applied to any factor. Therefore, factors were chosen that were considered most

important and most likely to vary in the use of Tedlar™ bags for disease diagnosis within a clinical environment, such as a hospital. These clinically viable factors included storage time, storage temperature, type of stored compounds, humidity, inter-bag variation and sample storage size. Factors such as emission of compounds from the bag material and the physical and chemical properties of the plastic would also be included when doing any tests.

Altering the concentration of the stored compounds was not tested. This choice was due to the limited scope of the project. Additionally, any methodology developed could be applied at a later stage to test the effect of compound concentration on the sample integrity of compounds stored in Tedlar™ bags.

4.2 COMPOUNDS, CONCENTRATION & SUBSTRATE

It was important to pick the substrate, compounds, and compound concentration to best test the integrity of breath samples stored in Teldar™ bags.

4.3 COMPOUNDS

To best test of the integrity of breath samples stored in Tedlar™ bags, the compounds used should cover a range of possible behaviour and be promising markers for breath diagnosis. A compound's behaviour is largely influenced by its volatility, polarity, size and functional group.

A good measure of a compounds size is the molecular mass with larger compounds generally having a larger mass. The molecular mass of the compound is the sum of all the atomic masses of the component atoms that make up the compound. The mass and

size of a compound affects its interaction with other entities, such as the bag wall, with smaller compounds being able to permeate through the bag. A compound's mass also affects its chemical reaction with other compounds as larger compounds are less likely to react. The molecular mass of the compound is also linked to volatility. Bigger compounds tend to have lower volatilities. SIFT-MS is limited to measuring compounds bellow 300 amu due to low volatilities of these heavier compounds.

Functional groups are specific groups of atoms within compounds. Examples of functional groups are ketones, alcohols and amines. The functional group is responsible for the characteristic chemical reactions polarity of the compound, which all affect its behaviour.

Polarity is a separation of charge and arises due to differences in the sharing of electrons between atoms and the asymmetry of the compound's structure. Often, one atom is more electronegative than its bonded counterpart(s), meaning shared electrons spend more time closer to the atom with the higher electronegativity. The inequal sharing of electrons between atoms creates both a positive and a negatively charged end of the compound. It is this difference in charge distribution that facilitates bonding with other like compounds with a similar charge distribution. Compounds with a separation of charge are termed polar, while those that have no charge separation are termed non-polar. One particularly strong form of polarity, for example, arises from hydrogen bonding.

Hydrogen bonding is a type of attractive intermolecular force involving a hydrogen atom attached to one of the elements oxygen, nitrogen or fluorine. As with standard

polarity, it arises due to differences in the electronegativites of bonded atoms. However, the difference in electronegativity is comparatively larger because hydrogen is at the extreme opposite end of electronegativity than oxygen, nitrogen or fluorine. Hence, compounds that have hydrogen bonds are very polar.

Polar compounds interact readily with water. When a compound is present in the gas phase, a measure of its interaction with water is its solubility which can be determined from Henry's law. Henry's law describes the relationship between gas solubility and pressure and is defined (Chang, 1998):

$$c = k_H P \qquad (4.1)$$

Where:

c = molar concentration in the liquid ($molL^{-1}$)

P = partial pressure (atm)

k_H = Henry's constant (mol/Latm)

Henry's constant is a good indication of a compounds readiness to dissolve in a liquid, such as water, when it is present as a gas above the liquid. The amount of gaseous compound that moves into a liquid increases with increasing k_H. Therefore, of two compounds present as a gas above a liquid, the one with the higher k_H will have a higher concentration in the liquid phase compared to another compound. The solubility of gases in water usually decreases with increasing temperature due to thermal energy transferred to the molecules, energising them to leave the liquid. Henry's constant for a given temperature is thus defined (Sander, 1999):

$$k_H = k_H^{\Theta} \times \exp\left(\frac{-\Delta_{so\ln} H}{R}\left(\frac{1}{T} - \frac{1}{T^{\Theta}}\right)\right) \qquad (4.2)$$

Where:

T^{Θ}	=	298 K
T	=	temperature of interest (K)
k_H^{Θ}	=	Henry's coefficient at 298 K
R	=	universal gas constant
$\Delta_{so\ln} H$	=	enthalpy of solution
$\frac{-\Delta_{so\ln} H}{R}$	=	$\frac{-d \ln k_H}{d(1/T)}$

It should be noted that for some compounds, such as ammonia, the dissolved gas reacts with water meaning higher solubilities can result.

Volatility is a measure of how rapid a chemical compound evaporates to the gaseous phase from a solid (sublimation) or liquid, and can be measured using the vapor pressure. Vapor pressure is the pressure of a vapor in equilibrium with its non-vapor phases at a given temperature. The higher the vapor pressure of a material, the more volatile it is. The more volatile a compound, the more likely it will be present in gaseous form and hence its behavior is affected.

4.3.1 SUBSTRATE

The substrate is the matrix in which the chosen compounds will reside. The substrate chosen should accurately reflect breath to ensure the measured sample integrity is

applicable to breath samples. The most important aspect of the substrate is that it is initially at 6% absolute humidity (100% relative humidity) and is non heterogeneous.

4.3.2 CONCENTRATION

There are a number of considerations for choosing a concentration level for the compounds. Firstly, the compound concentration should be similar to the concentration found in breath. Secondly, if breath is used as the substrate, there will be measurable levels of the chosen compounds present in the breath. These measured breath levels will add to any amount injected. To minimise any effects of pre-existing compound levels they should be at comparatively low concentration levels to those injected. To ensure comparatively low breath levels, they should be initially measured and the injected levels then selected. To draw conclusions on how compound type affects sample integrity it is also important that the compounds are at a similar starting concentration irrespective of their relative concentrations in actual breath.

4.3.3 PREVIOUS LITERATURE & STUDIES

Previous studies on the integrity of gas samples stored in plastic bags used a number of compounds at varying concentrations and in a number of different substrates, as shown in Table 4.1. From Table 4.1, the most common analyte used in prior research was methanol. The only important breath metabolites tested included acetone (Posner & Woodfin, 1986; Groves & Zellers, 1996) and ammonia (Spanel, Davies, & Smith, 1998).

Substrates were generally dry air or nitrogen. Spanel et al (1998) used breath as a substrate and Groves et al (1996) used air at breath humidity levels. The

concentrations were generally much higher than those found in breath. Therefore, there was no sample integrity study found that used all the common breath analytes (ammonia, ethanol, isoprene, acetone, pentane) at common breath levels (1-2ppm) and in a suitable substrate (breath or an approximation of breath).

Ref	Compounds (level in ppm)	Substrate	Bag Type(s)	Testing duration	Test Machine
1	benzene (10 -50) methanol (100-400) methyl isobutyl ketone (50-200) methylene chloride (250-650)	dry air	Saran, Scothpak	5 days	GC-MS
2	carbon monoxide (9) non-methane hydrocarbons (9) methane (9)	dry air	PVC, Tedlar, Snout, aluminized polyester	24,48,100 hours	GC-MS
3	acetone (100-700) benzene (107) 1,3-Butadiene (9.5) 1-butene (1050) methanol (200) 1,1,1-tricholoroethylene (100)	dry air & Nitrogen	Saran, Teflon, aluminized polyester Halar,	0,4,6,24,168 hours	GC-MS
4	methanol (11-40) formaldehyde (11-40)	exhaust fumes	Tedlar	6,24 hours	GC-MS
5	methanol (40) acetone (20) 2-butanone (8) perchloroethylene (16) 1,1,1-tricholoroethylene (9) m-xylene (4)	humid air	Tedlar	70min	GC-MS
6	ammonia (0.2-1.75)	breath	Tedlar	6 hours	SIFT-MS
7	styrene (90) allyl-alcahol (90) ehtylbenzene (90) propylene oxide (90) methyl tert-butyl ether (90)	dry air	Tedlar	3 weeks	GC-MS

Table 4.1: Compounds used in past literature including their concentration levels and substrate

(1) Smith & Pierce, 1970 (2) Polasek & Bullin, 1978 (3) Posner & Woodfin, 1986
(4) Andino & Butler, 1991 (5) Groves & Zellers, 1996 (6) McGarvey & Shorten, 2000

4.3.4 SELECTION OF COMPOUNDS, CONCENTRATION & SUBSTRATE

In order to establish the integrity of breath samples stored in Tedlar™ bags, ammonia, acetone, ethanol, isoprene and pentane were selected. These compounds are the most common and abundant breath metabolites and are promising markers of a number of diseases. The compounds also cover a range of functional groups, volatility and molecular masses, and involve non-polar and polar compounds meaning that a range

of possible compound behaviour will be represented. Covering a range of volatilities, polarities, masses and functional groups will rigorously test the Tedlar™ bag sample integrity, as it is known that highly polar compounds adhere to the inner bag surface and low molecular weight compounds may permeate through the Tedlar™ bag. The compounds chosen will also not react with each other, or any other compounds previously existing in the breath substrate. A summary of the compounds chosen is shown in Table 4.2

The substrate chosen was breath. Using breath as a substrate will ensure the tested sample integrity is applicable to breath samples. Creating substrates to accurately reflect breath is also more complex and labour intensive compared to filling a Tedlar™ bag with breath. Results from non-breath substrates may not be applicable to actual breath samples.

The mean concentration in breath of each chosen compound, as discussed in chapter 1, is: ammonia, 422–2389 ppb; acetone, 293–2000 ppb; isoprene, 55–600 pbb; ethanol, 27–1000 ppb; pentane 6-21 ppb in breath. The chosen concentration level was therefore 3ppm. A concentration level of 3ppm is considerably higher than the concentration levels of a healthy normal subject, as shown in Table 4.3, whose breath could be used as the substrate. The concentration level of 3ppm should therefore not be affected by existing concentration levels in the breath substrate. A concentration of 3ppm is also still an appropriate concentration to be representative of actual breath samples.

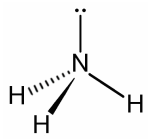
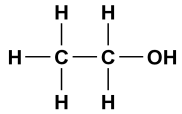
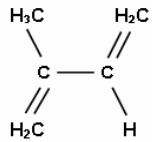
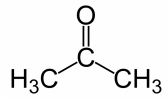
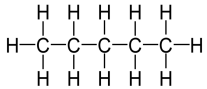
Compound	Fromula	Functional Group	Henry's Constant (mol/Latm)			Polarity	Hydrogen Boding	Molecular Mass (amu)
			22℃	37℃	25℃			
Ammonia 	NH_3	Amine	67	34	59	Polar	Yes	17
Ethanol 	C_2H_5OH	Alcahol	240	80	190	Polar	Yes	46
Isoprene 	C_5H_8	Alkene	N/a	N/a	0.013	Non-Polar	No	68
Acetone 	CH_3COCH_3	Ketone	35	16	30	Polar	No	58
Pentane 	C_5H_{12}	Alkane	N/a	N/a	0.0008	Non-Polar	No	72

Table 4.2: Compound information, Henrys law constants taken from (Sander, 1999) and molecular masses taken from (Chang, 1998)

Compound	Concentration (ppm)
Pentane	10-20
Ammonia	200-400
Isoprene	200-350
Ethanol	300-400

Table 4.3: Concentration levels of pentane, ammonia, isoprene and ethanol from a healthy normal subject whose breath could be used as the substrate

4.4 STORAGE TIME

The time from sample collection to analysis is mainly dependent on the proximity of a SIFT-MS instrument to the collection location. It will also be affected by the sampling routine used. If samples are collected close to a SIFT-MS they may be analysed within minutes, but otherwise may not be analysed for many hours or even days. The storage time was therefore chosen to be within 48 hours. To cover all storage time possibilities, the testing times were staggered closely at the start and then more widely towards the end.

4.5 STORAGE TEMPERATURE

Two storage temperatures were chosen to show the effect of storage temperature on the sample integrity of breath samples stored in Tedlar™ bags. Room temperature was chosen as it would not require incubation of samples and therefore be the most ideal storage temperature. Body temperature, 37°C, was also chosen as it represented the temperature breath would enter the SIFT-MS as a direct breath test.

4.6 HUMIDITY

Breath is at 6% absolute humidity (100% relative humidity) when it leaves the body. The humidity of samples affects two important experimental aspects, the compound's behaviour and the analysis using SIFT-MS. Humidity also affects compounds

differently, depending on their functional group and polarity. It was important to know whether the humidity was a reason for the sample integrity behaviour observed. Humidity also affects the analysis of samples using SIFT-MS, in particular, the calculation of the concentration. Humidity adds complexity to the analysis, involving water clusters and other associated chemistry. Therefore, a low humidity sample would also indicate whether the analysis method used by SIFT-MS was adequate.

Therefore, the humidity of samples was monitored and another set of Tedlar™ bags would be used with dry Nitrogen as the substrate. These tests cover the effect of humidity on both sample integrity, as well as the analysis technique used by SIFT-MS. Hence they represent a form of control.

4.7 INTER-BAG VARIATION

An important aspect of the integrity of breath samples stored in Tedlar™ bags was how the sample integrity altered between bags under the same conditions. To observe any inter-bag variation in the sample integrity, each testing situation (storage temperature, bag size and substrate) was performed with triplicate bags. Therefore, each testing situation will have three identical bags that will be sampled at the same time and manner over the storage duration.

4.8 TEDLAR™ BAG VOLUME AT SAMPLING PERIOD

It is possible to choose the bag testing method so that the volume of the Tedlar™ bag is either constant or different at each sampling period. To have a constant volume, samples could be taken once from different bags, each of which is filled to the same volume at each time period. The bags would then be discarded. Sampling from a

different volume could involve taking sequential samples at each sampling period from the same bag, reducing the volume of that bag over time.

Sampling from a constant volume is more appropriate to what would occur in clinical reality as samples would be filled and left until tested. However, sampling from a constant volume requires many bags to be made up. This approach thus introduces error, with differences in filling the bags to the same volume and spiking them to achieve the same concentration. Sequentially sampling from a bag also reduces the volume to surface area, which is one of the possible variables that could affect the integrity of samples. Therefore, sampling from a changing volume was carried out in this experiment and thesis.

4.9 SAMPLE STORAGE SIZE

Tedlar™ bags are commercially available in three main sizes: 0.5,1 and 3 L. The choice of sample storage size is reliant on the volume of sample required for analysis by SIFT-MS. It is also a function of the clinical practicality of obtaining a given sample volume from a patient both physically and within a feasible time frame. Therefore, the three commercially available storage sizes 0.5, 1 and 3L were all examined.

4.10 SUMMARY

The methodology aim included testing the storage time, storage temperature, type of stored compounds, humidity, inter-bag variation and stored sample size.

Ammonia, acetone, ethanol, isoprene and pentane were the chosen compounds. These compounds are common breath compounds and are promising markers of a number of

diseases. The compounds also cover a range of functional groups, volatilities and molecular masses. They also involve non-polar and polar compounds, meaning that a range of possible compound behaviour will be represented. The chosen concentration level was 3ppm, which is comparatively higher than existing breath levels while still being a representative level of actual breath samples.

The storage time was chosen to be within 48 hours. Two storage temperatures were chosen, room temperature and 37°C. Both dry Nitrogen substrates and humid breath substrates will be tested and the humidity monitored. To observe any inter-bag variation in the sample integrity, each testing situation will have triplicate bags. Sequentially sampling from a bag will be done as it also tests the effect of surface area to volume ratio. The three commercially available storage sizes 0.5, 1 and 3L will also be used.

This chapter has discussed the factors which affect the integrity of samples stored in Tedlar™ bags and incorporated them into a methodology and aim. The next chapter will describe the experimental method which was part of the methodology and will be implemented to test the aim developed.

4.11 REFERENCES

Andino, J. M., & Butler, J. W. (1991). A Study of the Stability of Methanol-Fueled Vehicle Emissions in Tedlar™ Bags. *Environ. Sci. Technol, 25*, 1644-1646.

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5 EXPERIMENTAL SETUP

An experiment was developed as part of the methodology for establishing the sample integrity of breath stored in Tedlar™ bags. The aim of the experiment was to test the effect of storage time, storage temperature, sample storage size (surface area to volume ratio) and sample humidity on the sample integrity of ammonia, acetone, isoprene, pentane and ethanol in Tedlar™ bags, and to obtain an estimate of the inter-bag variation of the sample integrity. These are the primary variables and compounds often encountered in many clinical breath testing studies.

It was not possible to investigate all the effects and compounds using a single test due to the physical resources and time required, and certain aspects of the analysis. Because the product mass 53 from reactions with the O_2^+ precursor overlapped for ammonia and isoprene they could not be simultaneously monitored at the same concentration level, and therefore required separate tests. Because ammonia was anticipated to be the most variable, it was tested separately from the other compounds. Therefore, two sets of experiments were performed, one with only ammonia and the other with pentane, isoprene, ethanol and acetone in Tedlar™ bags. The surface area to volume ratio was also explicitly tested by altering the bag size through the commercially available sizes. Only pentane, isoprene, ethanol, acetone in breath would be used. Because the behaviour of water was an excellent test of the surface

area to volume ratio, no dry nitrogen substrate was used. In all cases, three bags at each storage condition were used to test inter-bag variation. Therefore, three experiments were undertaken.

1 Effect of storage sizes: 0.5, 1, 3L on the sample integrity of pentane, isoprene, ethanol, acetone in breath and stored at 23-25°C and 37°C. Eighteen bags were tested in total, as shown in Figure 5.1.

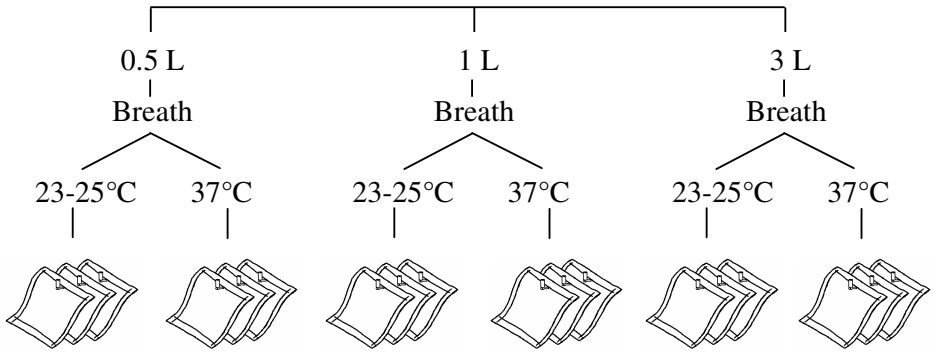

Figure 5.1: Experimental layout for sample integrity test 1

2 Sample integrity of ammonia in breath and nitrogen and stored at 23-25°C and 37°C using 1L Tedlar™ bags.

3 Sample integrity of pentane, isoprene, ethanol, acetone in breath and nitrogen in breath and nitrogen and stored at 23-25°C and 37°C using 1L Tedlar™ bags.

For both experiments 2 and 3, twelve bags in total were tested as shown in Figure 5.2.

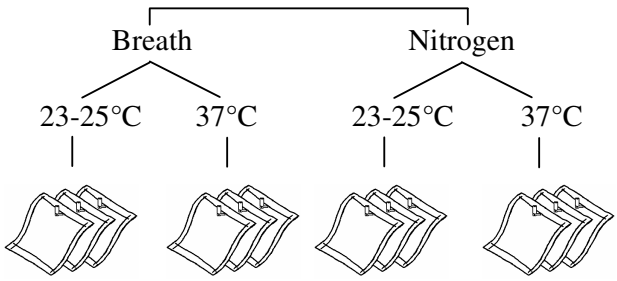

Figure 5.2: Experimental layout for sample integrity of tests 2 and 3

The three tests in each case had identical procedures of bag filling, bag spiking, and storage of the bags.

5.1 BAG FILLING & SPIKING

Tedlar™ bags were filled with the substrate and spiked with calculated amounts of analyte to give the desired concentration. The amount of analyte required to create a level of 3 ppm within the bag was dependent on the volume of the bag. It was therefore important to ensure that the filling method was consistent and accurate.

5.1.1 BAG FILLING

Two filling methods were used to test the consistency of filling Tedlar™ bags. First, the bags were filled and then left to slightly deflate naturally. The volume was calculated by measuring the time for a Gilian LFS-113 constant flow rate pump to empty the bag (0.5 $Lmin^{-1}$). It was found that this filling method gave reasonably constant volumes for each bag, but between bags gave differences of around 100mL. The inter-bag volume variation was due to differences in the shape the bags took

when left to deflate naturally. The second filling method was to fill the bags until they were firm but not rigid. As shown in Table 5.1, this filling method ensured a consistent volume both between bags and within each bag.

Bag	Test	Volume (L)
1	1	1.0364
	2	1.0716
	3	1.0584
	4	1.0496
2	1	1.0012
	2	1.0144
	3	1.0232
	4	0.9748
3	1	1.0716
	2	1.076
	3	1.054
	4	1.076

Table 5.1: Consistency of bag filling volumes

To create the breath substrate, the person providing it exhaled through a straw for 5 seconds and then exhaled into a Tedlar™ bag through the side port as shown in Figure 5.3. This approach was used to ensure the bag contained humid breath and not ambient air from the airways deadspace. For the nitrogen substrate, Tedlar™ bags were filled from a cylinder containing analytical grade nitrogen.

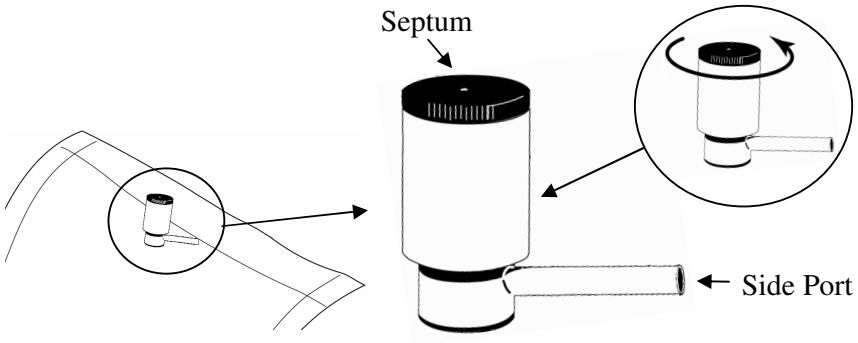

Figure 5.3: Tedlar™ bag septum and side port which can be sampled after turning the head

5.1.2 SPIKING

Samples of the compounds were made up in 1L glass bulbs to give 10000 ppm or 1% concentrations. The glass bulbs had a pierceable rubber septum at their base. A gastight syringe was used to transfer calculated amounts of a compound from the glass bulb to the Tedlar™ bags. The gastight syringe used was a 1mL Hamilton™. The amount of compound required to add to the Tedlar™ bags to give a concentration of 3ppm is shown in Table 5.2.

Tedlar bag volume (L)	Volume added to Tedlar bag from %1 glass bulb (mL)
3	0.9
1	0.3
0.5	0.15

Table 5.2: Volume added from a %1 glass bulb sample to give 3ppm in a given Tedlar™ bag size

Each Tedlar™ bag was filled with either breath or nitrogen. Using the 1mL Hamilton™ gas tight syringe the appropriate amount of each analyte to be monitored was extracted from the appropriate glass bulb and injected into the Tedlar™ bag through its septum. An example of a three compound spiking to give three identical Tedlar™ bags is shown in Figure 5.4.

Samples taken from each 1L glass bulb accounted for 0.03% of the total bulb volume. The maximum number of bags to be spiked was the 18 bags shown in Figure 5.1 for the effect of storage size tests. Spiking the 18 bags removed 8.1mL or 0.81% of the total bulb volume. This small decrease in bulb volume and resulting decrease in bulb concentration was deemed to be negligible. Similarly, the small volume added to the Tedlar™ bags accounted for approximately 0.03 % of the total bag volume. Therefore, the injected amount did not significantly increase the volume of the bag

and therefore did not need to be taken into account when calculating the volume to be injected.

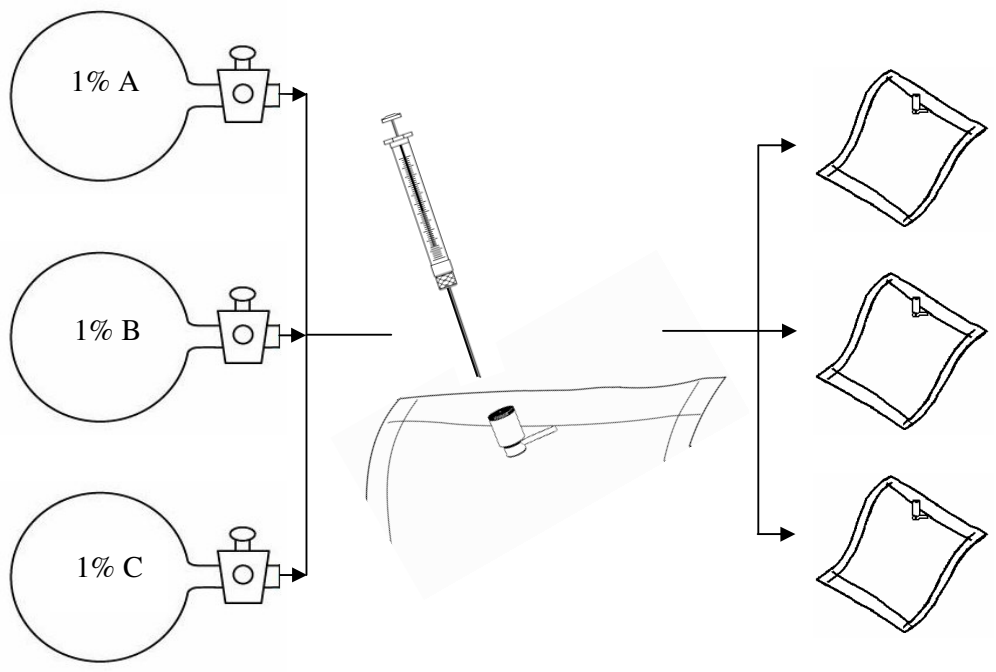

Figure 5.4: Spiking Tedlar™ bag samples with three compounds by injecting through the septum

5.2 TEST MACHINE

The SIFT-MS instrument used for testing was LDI2 (Lab Demonstration Instrument 2) located at Christchurch Hospital. The capillary flow rate of LDI2 is between 2.1-2.5 torrLs^{-1} (2.8mLs^{-1} - 3.3mLs^{-1}). It was used for all tests described in this thesis.

5.3 STORAGE OF BAGS

Room temperature (23°C- 25°C) and 37°C were the storage temperatures tested. The 23-25°C stored bags were stored in the same location as LDI2. The 37°C stored bags were kept in an incubator. The incubator was accurate to within 0.1°C. The 37°C stored samples would be removed from the incubator for testing and would therefore

be exposed to room temperatures (23°C- 25°C). To prevent cooling of the bags a transport incubator was constructed. The transport incubator fitted a single bag and allowed the bag to be tested while still being heated within the incubator. In this way, the bags were maintained at 37°C during transport to LDI2 and testing. The transport incubator was accurate to within 2°C. In both the 23-25°C and 37°C storage temperatures, the temperature was monitored and controlled to ensure it remained constant.

5.4 TESTING TIMES AND SAMPLE PERIOD

Using a 20 second sampling period and the LDI2 capillary sampling rate of 3.3mLs^{-1}, a maximum of 8 samples could be taken from a 500mL bag, 15 from a 1L bag, and 45 from a 3L bag. The effect of storage size tests were carried out over a 48 hour period. However, only the sample integrity over the first 4 hours would be studied. The testing times were:

500mL - 10:00, 10:30, 11:30, 1:30, next day 10:00, 4:00, next day after 10:00

1L & 3L - 10:00, 10:30, 11:00, 12:00, 1:00, 2:00, next day 10:00, 2:00, next day after 10:00

The other two tests were carried out over a 6 hour period. The testing times were: 10:00, 10:20, 10:40, 11:00, 11:20, 11:40, 12:00, 1:00, 2:00, 3:00, 4:00.

5.5 TEST PROCEDURE

1 Remove 37°C stored bag from the incubator place into the transport incubator or take 23-25°C stored bag from its container. Attach bag to the LDI2 inlet and open the side port. The bags are sampled using the side port and not the septum because using the septum can cause leakage.

2 Directly test each bag using a 20 second SIM scan for all monitored analytes. The water and cluster masses are also automatically monitored for the humidity calculation.

3 Place bag back into incubator or storage location after the SIM scan

4 Repeat until all bags are tested

Note that at least two runs of each experiment are performed to ensure and quantify the reproducibility of the results.

5.6 INSTRUMENT REPEATABILITY AND PRECISION

No study on the repeatability of SIFT-MS has been reported to date. Therefore, it was important to gain a measure of the repeatability and precision of LDI2. The repeatability and precision are most likely dissimilar for different compounds, precursors used and concentrations. It was not within the scope of this thesis to fully establish and validate the precision and repeatability of SIFT-MS instruments, but rather to obtain a reasonable estimate to achieve the end goals of the research presented.

To gain the best estimate of the repeatability and precision of LDI2, a cylinder containing constant amounts of acetone, pentane and ethanol was be tested seven times over a 250 min time period using a 25 point sample. Due to the way the cylinder is made, it is not possible to know the concentration of the compounds in the cylinder, however, they will be constant. To remove any variability caused by water and sample line losses the cylinder was be dry (0.05% absolute humidity) and be plumbed directly into the inlet to LDI2.

Acetone, pentane and ethanol were chosen as they are representative of the range of compounds tested. They include a range of monitored masses including the total number monitored for each compound and involve all the precursors. These compounds also cover a range of functional groups, volatility and molecular masses, and involve non-polar and polar compounds. Hence, a broad range of possible compound behaviour is covered.

5.7 BACKGROUND PERMEATION TEST

Due to the chemical structure of Tedlar™, low molecular weight compounds may permeate the bag (Environmental Response Team, 2003; McGarvey & Shorten, 2000). It was therefore important to monitor the storage environment for levels of the tested compounds that may contaminate the bags. A 1L bag filled with nitrogen was placed in the room and incubator storage locations. The bags were tested for all monitored compounds before and after the final storage time.

5.8 SUMMARY

Four experiments were developed to test the effect of storage time (6 - 48 hours), storage temperature (23°C- 25°C, 37°C), storage size (0.5, 1, 3L) and sample humidity (breath and nitrogen substrates) on the sample integrity of ammonia, acetone, isoprene, pentane and ethanol in Tedlar™ bags. An estimate of the inter-bag variation of the sample integrity was also established by having triplicate bags for each storage condition (temperature, substrate, storage size).

The precision and repeatability of LDI2 was established by testing a cylinder containing constant amounts of ethanol, pentane and acetone. A background permeation test using a 1L nitrogen filled Tedlar™ bag was used to indicate significant permeation of compounds from the atmosphere into samples.

To create samples, Tedlar™ bags were filled with the substrate and spiked from glass bulbs with calculated amounts of analyte to give 3ppm. The 37°C stored bags were kept in an incubator and transported and heated during testing using a transport incubator. A 20 second sampling period was used.

This chapter described the experimental method developed as part of the methodology for establishing the integrity of breath samples stored in Tedlar™ bags and the machine repeatability and precision. The next chapter will describe the analysis methods used to best indicate the integrity of samples stored in Tedlar™ Bags and the machine repeatability and precision.

5.9 REFERENCES

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6 ANALYSIS METHOD

The analysis methods developed utilised the SIFT-MS instrument measurements and their treatment to best indicate the integrity of samples stored in Tedlar™ Bags. This analysis method therefore comprised: the kinetics, sample statistical analysis and software written to carry out the analysis.

6.1 KINETICS

SIFT-MS measures the count rates for a range of masses, which are then identified as either precursor or product masses, and then used to calculate the concentration of compounds. The choice of precursor and product masses including the reaction rate coefficients and branching ratios, make up the kinetics. An important aspect of the kinetics are the reaction rate coefficients.

As discussed in chapter 2, the reaction rate coefficients describe the speed at which reactions occur and link the rate of product formation with reactants. Reaction rate coefficients are not empirical factors and therefore it is said that SIFT-MS does not need to be calibrated. The reaction rate coefficients can be found using two methods, absolute methods and relative methods (Syft, 2005). A form of the absolute method was

implemented to find the reaction rate coefficients used in the analysis by Syft Technologies using LDI2.

The method used was a machine calibration and was therefore specific to LDI2. The rate coefficients used are thus, in this sense, empirical factors, and therefore not transferable between different types of SIFT-MS instruments. Comparing the reaction rates used to the theoretical collision limiting rates gives an idea of how much the reaction rates were altered. In most cases the reaction rate coefficients were only altered by slight amounts and were of the same order, as can be seen in Table 6.1. However, it should be noted that the choice of the monitored products, including their branching ratios are generally common in all kinetics and are therefore effectively universal.

The calibration method used to find the rate coefficients involved spiking 1L Tedlar™ bags with known amounts of analyte into nitrogen and breath substrates to give three concentrations at approximately 3, 5, 10 ppm. Bags were tested at room temperature (23°C-25°C). The reaction rate coefficients were adjusted until the concentrations in each substrate were linear and agreed over the tested concentration range when the same kinetics were used. An example of a calibration run for ethanol is shown in Figure 6.1, while the calibration runs for acetone, isoprene, ammonia and pentane are located in the Appendix A6.

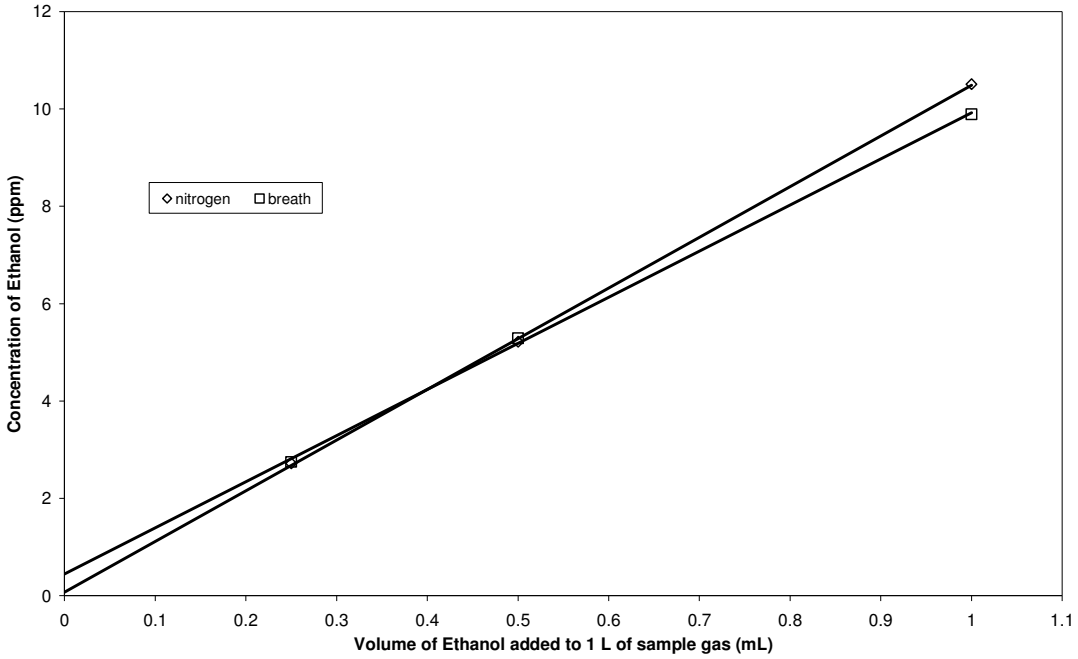

Figure 6.1: Calibration performed for ethanol in nitrogen and breath substrates using LDI2

The calibration method could not be performed for ammonia in a breath substrate due to concentration fluctuations. Ammonia was therefore only calibrated for the nitrogen substrate. For acetone, different masses were monitored depending on whether the substrate was humid or dry, as shown in Table 6.1 and 6.2.

Masses monitored in the humid condition were those resulting from secondary reactions with water. These secondary reactions with water would be negligible in dry substrates and hence could be ignored. A summary of the kinetics used in the analysis, which includes the monitored product masses for each precursor and compound including their branching ratios, product order and rate coefficients, is also shown in Table 6.1 and 6.2.

Compound	Precursor	Mass (amu)	br	Order	k (×10^{-9})	k_{col} (×10^{-9})
Acetone	H_3O^+	19			4.1	3.9
		37			3	3.2
		55			2.5	2.8
		73			2.2	2.4
		59	1	P		
		77 (humid)		S		
		95 (humid)		S		
	NO^+	30			1.5	3.3
		88	1	P		
Isoprene	O_2^+	32			1.3	1.6
		68	0.45	P		
		67	0.45	P		
		53	0.1	P		
	H_3O^+	19			2.2	2
		37			1.7	2
		55			9	2
		73			8	2
		69	1	P		
		87		S		
	NO^+	30			1.4	1.7
		48			1.4	1.7
		68	0.9	P		
		86		S		
		98	0.1	P		
Ethanol	H_3O^+	19			2.6	2.7
		37			2.6	2.7
		55			0.9	2.7
		73			0.6	2.7
		47				
		65				
		83				
	NO^+	30			0.8	2.3
		48			8	2.3
		45	1	P		
		63		S		
		81		S		
		99		S		
Pentane	O_2^+	32			1.2	1.6
		71				
		72				
Ammonia	H_3O^+	19			1.7	2.71
		37			1.3	2.71
		55			0.88	2.71
		73			5.5	2.71
		18	1	P		
		36		S		
		54		S		
	O_2^+	32			1.1	2.4
		17	0.7	P		
		35		S		
		53		S		
		18	0.3	P		
		36		S		
		54		S		

Table 6.1: Kinetics used in analysis, P = primary and S = secondary products, br = branching ratio, all masses monitored for dry and humid conditions unless stated

Precursor	H_3O^+	NO^+	O_2^+
common to all compounds	19 37 55 73	30 48	32
Acetone	59 77 (humid) 95 (humid)	88	
Isoprene	69 87	68 86 98	53 67 68
Ethanol	47 65 83	45 63 81 99	
Pentane			71 72
Ammonia	18 36 54		17 18 35 36 53 54

Table 6.2: Monitored masses (amu) for each precursor and compound

6.1.1 OVERLAPPING MASSES

It is important, when using the same precursor, that any masses used to measure a product for a compound do not overlap with a product mass monitored for any other compound. If overlaps occur, the count rates would involve counts from both products and result in incorrect concentrations. It should be noted that mass overlaps between precursors is not problematic, as only one precursor will be present in the flow tube at any time. However, preventing overlapping masses does have implications on the analysis and kinetics used.

Both ammonia and isoprene measure mass 53 when using the O_2^+ precursor, as shown in Table 6.2. Therefore, when ammonia and isoprene are present in a sample and monitored with the O_2^+ precursor, there will be overlapping of the 53 product mass. As the breath

substrate has ammonia and isoprene present, there is always overlapping of the mass 53. The effect of overlapping is diminished when the overlapping mass does not contribute greatly to the total products counted for a compound or when the measured compound is at a comparatively higher concentration than the compound which it overlaps. These two situations are applicable to ammonia and isoprene. For ammonia, the 53 product mass is produced from clustering of the ammonia ion with water to produce $NH_3^+.(H_2O)_2$ as shown in Appendix A5. This is a secondary reaction and therefore has minimal contribution to the products. For isoprene, the product mass of 53, which occurs from reactions with the O_2^+ precursor, is a primary product with a low branching ratio of 0.1, as shown in Table 6.1. Therefore, the product mass contributes 10 % to the total counted products. Overall, for both ammonia and isoprene, the 53 product mass does not make significant contributions to the total products.

In addition, the concentrations of ammonia and isoprene are also comparatively different. The breath substrate contains approximately 200-350 ppb and 200-400 ppb of isoprene and ammonia respectively as shown in Table 4.3. The compound concentrations used in the experiments will be 3ppm. Therefore, the overlapping becomes less problematic by ensuring the concentration of ammonia and isoprene are significantly different.

Ideally, the monitoring of pentane would involve masses 42, 43, 57, 72, 71 and 97. These masses are common to many breath analytes and therefore, as with isoprene and ammonia, would be problematic due to overlapping masses when monitoring pentane and other breath compounds simultaneously. Therefore, only the 71 and 72 masses were

monitored and their rate coefficients were multiplied by factors to account for missing masses before calibration. Although there was no overlapping of the extra pentane masses with ammonia, isoprene, ethanol or acetone, only masses 71 and 72 would be monitored, as this approach comprises the kinetics developed and utilised by Syft Technologies.

6.1.2 TEDLAR™™ BAG CONTAMINANTS

Tedlar™ bags contain phenol and N,N-dimethylacetamide at levels of 200-500 ppb and 500-700 ppb, respectively. The product masses from reactions with precursors are shown in Table 3.3. The mass of 95 (phenol) overlaps with the monitored mass for acetone in humid substrates, which can be seen from comparing Table 6.2 and 6.3. However, as acetone will be present at 3ppm in these experiments, the relative concentrations of phenol and acetone ensure the contribution of phenol is insignificant. It is also not anticipated that phenol and N,N-dimethylacetamide, at their relevant concentrations will not react with, or be problematic for, analysis of the other compounds, specifically, ammonia, pentane, isoprene or ethanol.

Precursor	Phenol Mass (amu)	N,N-dimethylacetamide Mass (amu)
H_3O^+	95	88
O_2^+	94	87
NO^+	94	87, 117

Table 6.3: Product masses monitored from reaction of precursors with phenol and N,N-dimethylacetamide

6.2 STATISTICAL ANALYSIS

There are a number of important aspects in a methodology used to test the integrity of breath samples stored in Tedlar™ bags. To ensure the accuracy, robustness and repeatability of a given sample integrity for any given compound and storage conditions, tests should be repeated for a large number of bags. The results should then be combined to give an overall sample integrity within which any later sample integrity measurements should lie. The methodology should therefore be able to combine any given number of bags. In the experiments used to determine the sample integrity, the starting concentrations would be approximately the same but not identical. Having slightly different concentrations is representative of any disease where the limits could cover a range of concentrations and could be dependent on the patients size or age. Therefore, the methodology should be able to combine bags of different concentration levels. To obtain information on the sample integrity, the analysis method should thus indicate the mean and variance of the measured concentration, taking into account the machine variation of SIFT-MS within sampling periods. The method should also indicate any inter-bag variation.

In summary, the analysis method should be able to:

- combine multiple bags
- combine bags at different concentrations
- indicate a combined distribution including mean and variance
- indicate differences in inter-bag behaviour

6.2.1 NORMALISING CONCENTRATIONS

An initially obvious and simplistic approach was to normalise each Tedlar™ bag by their starting concentration. This approach was used by McGravey & Shorten (2000). Normalisation would make it easier to combine the compound sample integrity behaviour from different bags, which each had slightly different starting concentrations. It would also then be easier to compare the effect of different storage conditions and compounds, as each case would have the same starting value of 1. However, when normalising and combining the Tedlar™ bags, certain assumptions are made and aspects of the Tedlar™ bag behaviour are overlooked.

To consider normalising, it must be assumed the Tedlar™ bags have the same starting concentration. Although the starting concentrations were approximately the same in the experiments, they were not identical and not expected to be so. Hence, ideally they should not really be combined and treated as the same. When comparing normalised results it is assumed that the behaviour of the compounds is independent of their starting concentration. It therefore removes the possibility of concentration being the cause of the observed behaviour. Quantitative information on how differently the bags behave from one another over time is also lost. Therefore, using normalisation to combine the Tedlar™ bags is not useful in a methodology to determine the integrity of breath samples stored in Tedlar™ bags and does not properly describe the behaviour of the Tedlar™ bags. A more promising approach was to use a Bayesian analysis.

6.2.2 BAYESIAN ANALYSIS

For a given bag at a given time instant, assume the measurements taken over a set sampling period are normally distributed with mean μ and variance σ^2.

$$x_1,\ldots,x_n \quad \sim \quad N(\mu,\sigma^2) \qquad (6.1)$$

Where:

$x_1,\ldots,x_n$	=	measurements taken over the sampling period
μ	=	mean of measurements over sampling period
σ^2	=	variance of measurements over sampling period

It is reasonable to assume the measurements will be normally distributed by considering a typical SIM scan carried out by a SIFT-MS over a set sampling period. By plotting a histogram of the concentration points it can be seen that they form a normal distribution, as shown in Figure 6.2.

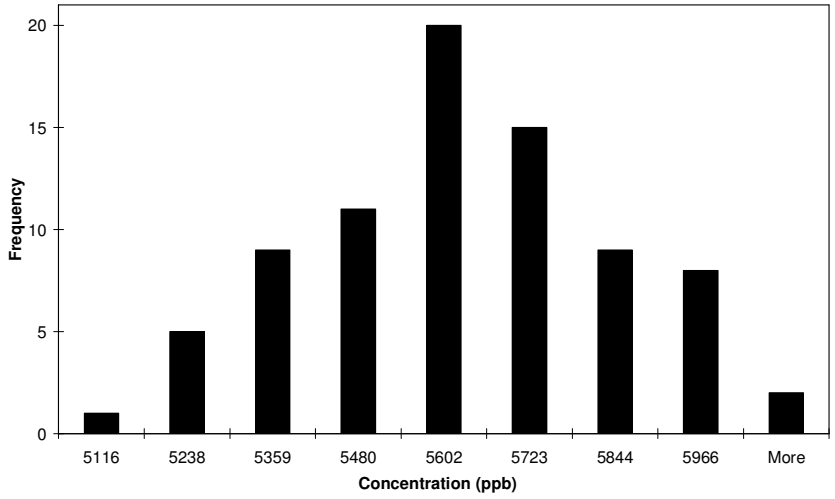

Figure 6.2: Histogram for an 80 point sample period indicating they are normally distributed

The joint normal density of the measurements $x_1,\ldots,x_n$ is given by:

$$f\left(x_1,\ldots,x_n \mid \mu,\sigma^2\right) \quad \propto \quad \left(\sigma^2\right)^{-n/2}\exp\left[-\frac{1}{2\sigma^2}\sum_{i=1}^{n}\left(x_i-\mu\right)^2\right] \qquad (6.2)$$

Letting $\hat{\mu}=\frac{1}{n}\sum_{i=1}^{n}x_i$ (sample mean) and $\hat{\sigma}^2=\frac{1}{n-1}\sum_{i=1}^{n}\left(x_i-\hat{\mu}\right)^2$ (sample variance) then:

$$\begin{aligned}\sum_{i=1}^{n}\left(x_i-\mu\right)^2 &= \sum_{i=1}^{n}\left(x_i-\hat{\mu}+\hat{\mu}-\mu\right)^2\\ &= \sum_{i=1}^{n}\left[\left(x_i-\hat{\mu}\right)^2+\left(\hat{\mu}-\mu\right)^2+2\left(x_i-\hat{\mu}\right)\left(\hat{\mu}-\mu\right)\right]\\ &= \sum_{i=1}^{n}\left(x_i-\hat{\mu}\right)^2+n\left(\hat{\mu}-\mu\right)^2+2\left(\hat{\mu}-\mu\right)\sum_{i=1}^{n}\left(x_i-\hat{\mu}\right)\\ &= (n-1)\hat{\sigma}^2+n\left(\mu-\hat{\mu}\right)^2 \quad \because\left(\sum_{i=1}^{n}\left(x_i-\hat{\mu}\right)=0\right)\end{aligned}$$

Therefore the likelihood function, in terms of μ and σ^2, can be expressed as:

$$f\left(x_1,\ldots,x_n \mid \mu,\sigma^2\right) \quad \propto \quad \left(\sigma^2\right)^{-n/2}\exp\left\{-\frac{1}{2\sigma^2}\left[(n-1)\hat{\sigma}^2+n\left(\mu-\hat{\mu}\right)^2\right]\right\} \qquad (6.3)$$

The assumed form of the distribution of μ and σ^2 before the data is obtained, i.e. the prior distribution is defined (Bernardo & Smith, 1994):

$$f\left(\mu,\sigma^2\right)\propto\left(\sigma^2\right)^{-1} \qquad (6.4)$$

The distribution of μ and σ^2 after obtaining the data, i.e. the posterior distribution, is defined:

$$f(\mu,\sigma^2 \mid x_1,\ldots,x_n) \propto f(x_1,\ldots,x_n \mid \mu,\sigma^2) f(\mu,\sigma^2) \quad \text{(Bayes Theorem)}$$

$$\propto (\sigma^2)^{-(n-2)/2} \exp\left\{-\frac{1}{2\sigma^2}\left[(n-1)\hat{\sigma}^2 + n(\mu-\hat{\mu})^2\right]\right\} \qquad (6.5)$$

Since the level of the readings is of interest, the posterior marginal distribution of μ is obtained by integrating Equation 6.5 with respect to σ^2.

$$f(\mu \mid x_1,\ldots,x_n) = \int f(\mu,\sigma^2 \mid x_1,\ldots,x_n) d\sigma^2$$

$$\propto \int (\sigma^2)^{-(n+2)/2} \exp\left\{-\frac{1}{2\sigma^2}\left[(n-1)\hat{\sigma}^2 + n(\mu-\hat{\mu})^2\right]\right\} d\sigma^2 \qquad (6.6)$$

The integral in Equation 6.6 is the normalising constant of the inverse gamma distribution (*IG*) with parameters:

$$IG\left(\frac{n}{2}, \frac{(n-1)\hat{\sigma}^2 + n(\mu-\hat{\mu})^2}{2}\right)$$

Therefore, the integral can be re-written:

$$\propto \left[\frac{1}{(n-1)\hat{\sigma}^2 + n(\mu-\hat{\mu})^2}\right]^{n/2}$$

$$\propto \quad \left[\left(\frac{n-1}{n}\right)\hat{\sigma}^2 + (\mu - \hat{\mu})^2\right]^{-n/2}$$

$$= \quad \left[\hat{s}^2 + (\mu - \hat{\mu})^2\right]^{-n/2} \qquad (6.7)$$

Where $\hat{s}^2 = \frac{1}{n}\sum_{i=1}^{n}(x_i - \hat{\mu})^2$

This is identified as a generalised Cauchy distribution. The generalised Cauchy distribution with parameters *a*, *b* and *m* is written as (Evans, Hastings, & Peacock):

$$GC(a,b,m) \quad = \quad \left[b^2 + (x-a)^2\right]^{-m} \qquad (6.8)$$

Comparing the form of Equation 6.7 to $GC(a,b,m)$ indicates that Equation 6.7 is proportional to the generalised Cauchy distribution. Therefore, $f(\mu \mid x_1,\ldots,x_n)$ is generalised Cauchy with parameters $\hat{\mu}$, $\hat{s}$, $n/2$.

The generalised Cauchy distribution can then be applied to multiple bags to give a combined distribution. For example, for three bags at a given time instant:

$$x_1,\ldots,x_{n_x} \quad \sim \quad N(\mu_x, {\sigma_x}^2) \qquad (6.9)$$

$$y_1,\ldots,y_{n_y} \quad \sim \quad N(\mu_y, {\sigma_y}^2) \qquad (6.10)$$

$$z_1,\ldots,z_{n_z} \quad \sim \quad N(\mu_z, {\sigma_z}^2) \qquad (6.11)$$

Where $x_1,\ldots,x_{n_x}$, $y_1,\ldots,y_{n_y}$, $z_1,\ldots,z_{n_z}$ are the three bag concentration measurements over the sampling period and are normally distributed with mean μ_x , μ_y , μ_z and variance σ_x^2, σ_y^2, σ_z^2 respectively.

Representing the mean level as GC:

$$f\left(\mu_x \mid x_1,\ldots,x_{n_x}\right) = GC\left(\hat{\mu}_x,\hat{s}_x,\frac{n_x}{2}\right) \quad \textbf{(6.12)}$$

$$f\left(\mu_y \mid x_1,\ldots,x_{n_y}\right) = GC\left(\hat{\mu}_y,\hat{s}_y,\frac{n_y}{2}\right) \quad \textbf{(6.13)}$$

$$f\left(\mu_z \mid x_1,\ldots,x_{n_z}\right) = GC\left(\hat{\mu}_z,\hat{s}_z,\frac{n_z}{2}\right) \quad \textbf{(6.14)}$$

The combined distribution for the mean level is defined as a weighted mixture distribution of the three individual GC distributions:

$$f(\mu \mid x,y,z) = \frac{n_x}{n}GC\left(\hat{\mu}_x,\hat{s}_x,\frac{n_x}{2}\right)+\frac{n_y}{n}GC\left(\hat{\mu}_y,\hat{s}_y,\frac{n_y}{2}\right)+\frac{n_z}{n}GC\left(\hat{\mu}_z,\hat{s}_z,\frac{n_z}{2}\right) \quad \textbf{(6.15)}$$

Where $n = n_x + n_y + n_z$

Equation 6.15 thus represents the combined distribution over all three bags. This approach can be generalised to any number of bags.

6.2.3 HUMIDITY

The humidity is not calculated and output by a SIFT-MS instrument during a SIM scan, but can be calculated based on measurements of selected water masses using Equation 2.26. Therefore, as single humidity values were not available, the variance and mean were calculated based on the formula used to find the humidity. Once the mean (μ) and variance (σ^2) were known, the combined humidity from the three bags could be described using the generalised Cauchy distribution.

A method for the calculation of absolute humidity using SIFT-MS was given by Spanel and Smith (2001).

$$[H_2O] = \frac{1}{t_r k_{2eff}} \ln \frac{\{H_3O^+\}D_{f1} + \{H_3O^+.H_2O\}D_{f2} + \{H_3O^+.(H_2O)_2\}D_{f3} + \{H_3O^+.(H_2O)_3\}D_{f4}}{\{H_3O^+\}D_{f1}} \qquad \textbf{(6 . 16)}$$

Where:

$\{H_2O\}$ = number density of water molecules (molecules cm^{-3})

t_r = reaction time (seconds)

$\{H_3O^+.(H_2O)_{0,1,2,3}\}$ = product or precursor ion counts (cps)

k_{2eff} = effective two-body rate coefficient(molecules cm^3 s^{-1})

D_{fi} = correction factor for mass discrimination and differential diffusion for the relevant mass (constants)

1 = $\{H_3O^+\}$; 2 = $\{H_3O^+.H_2O\}$; 3 = $\{H_3O^+.(H_2O)_2\}$;

4 = $\{H_3O^+.(H_2O)_3\}$

The absolute humidity, H_{AH}, can therefore be found from the H_3O^+. $(H_2O)_{0,1,2,3}$ ion count rates (Smith & Spanel, 2001):

$$H_{AH} = \frac{[H_2O]}{3.54\times10^{16}} \frac{T_g(\Phi_{He} + \Phi_{Ar} + \Phi_S)}{T_{ref}\Phi_S p_g} \qquad (6.17)$$

Where:

T_g = temperature of the gas in the flow tube (Kelvin, K)

p_g = flow tube pressure (torr)

T_{ref} = reference temperature for rate coefficients (273 K)

H_{AH} = absolute humidity (%)

Φ_S = sample flow through capillary (torr Ls^{-1})

$[H_2O]$ = number density of water molecules (molecules cm^{-3})

Φ_{He} = Helium Gas Flow Rate (torr Ls^{-1})

Φ_{Ar} = Argon Gas Flow Rate (torr Ls^{-1})

Φ_S = sample flow through capillary (torr Ls^{-1})

Defining the following terms:

Let $X_1 = \{H_3O^+\} D_{f1}$

$X_2 = \{H_3O^+.\ H_2O\} D_{f2}$

$X_3 = \{H_3O^+.\ (H_2O)_2\} D_{f3}$

$$X_4 = \{H_3O^+.(H_2O)_3\}\, D_{f4}$$

$$X_5 = \Phi_{He}$$

$$X_6 = \Phi_{Ar}$$

$$X_7 = \Phi_S$$

$$X_8 = p_g$$

This allows Equation 6.17 to be written as:

$$H_{AH} = \frac{T_g\left(X_5 + X_6 + X_7\right)}{3.54\times10^{16} t_r k_{2eff} T_{ref} X_7 X_8} \ln\left(\frac{X_1 + X_2 + X_3 + X_4}{X_1}\right) \quad \textbf{(6.18)}$$

Assume that $X_1, X_2 \ldots, X_8$ are independent random variables and that H_{AH} is a function of $X_1, X_2 \ldots, X_8$ which is reasonably well behaved, approximately linear and therefore close to their mean values $E(X_1), E(X_2) \ldots, E(X_8)$. Using the Delta method, a first order multivariate Taylor expansion is used to linearise H_{AH} with respect to each measured quantity. Therefore:

$$H_{AH}(X_1, X_2 \ldots, X_8) \approx H_{AH}(E(X_1), \ldots, E(X_8)) + \frac{\partial H_{AH}}{\partial X_1}(X_1 - E(X_1)) + \ldots + \frac{\partial H_{AH}}{\partial X_8}(X_8 - E(X_8)) \quad \textbf{(6.19)}$$

As it is assumed that $(X_1, X_2 \ldots, X_8) \approx (E(X_1), E(X_2) \ldots, E(X_8))$ then:

$$E\left[H_{AR}(X_1, X_2 \ldots, X_8)\right] \approx H_{AH}(E(X_1), E(X_2) \ldots, E(X_8)) \quad \textbf{(6.20)}$$

Therefore the mean of the absolute humidity, $E(H_{AR})$, and variance, $Var(H_{AR})$, are thus defined:

$$E(H_{AH}) \approx \frac{T_g\left(E(X_5)+E(X_6)+E(X_7)\right)}{3.54\times10^{16}t_r k_{2eff} T_{ref} E(X_7)E(X_8)} \ln\left(\frac{E(X_1)+E(X_2)+E(X_3)+E(X_4)}{E(X_1)}\right) \quad \textbf{(6.21)}$$

$$Var\left[H_{AR}(X_1,\ldots,X_8)\right] \approx \left(\frac{\partial H_{AH}}{\partial X_1}\right)^2 Var(X_1)+\ldots+\left(\frac{\partial H_{AH}}{\partial X_8}\right)^2 Var(X_8) \quad \textbf{(6.22)}$$

Where:

$$\frac{\partial H_{AH}}{\partial X_1} = \frac{T_g\left(\frac{1}{X_1}-\frac{X_1+X_2+X_3+X_4}{{X_1}^2}\right)X_1\left(X_5+X_6+X_7\right)}{3.54\times10^{16}t_r k_{2eff} T_{ref}\left(X_1+X_2+X_3+X_4\right)X_7X_8} \quad \textbf{(6.23)}$$

$$\frac{\partial H_{AH}}{\partial X_2},\frac{\partial H_{AH}}{\partial X_3},\frac{\partial H_{AH}}{\partial X_4} = \frac{T_g X_1\left(X_5+X_6+X_7\right)}{3.54\times10^{16}t_r k_{2eff} T_{ref}\left(X_1+X_2+X_3+X_4\right)X_7X_8} \quad \textbf{(6.24)}$$

$$\frac{\partial H_{AH}}{\partial X_5},\frac{\partial H_{AH}}{\partial X_6} = \frac{T_g \ln\left(\frac{X_1+X_2+X_3+X_4}{X_1}\right)}{3.54\times10^{16}t_r k_{2eff} T_{ref} X_7X_8} \quad \textbf{(6.25)}$$

$$\frac{\partial H_{AH}}{\partial X_7} = \frac{T_g \ln\left(\frac{X_1+X_2+X_3+X_4}{X_1}\right)}{3.54\times10^{16}t_r k_{2eff} T_{ref} X_7X_8} - \frac{T_g \ln\left(\frac{X_1+X_2+X_3+X_4}{X_1}\right)\left(X_5+X_6+X_7\right)}{3.54\times10^{16}t_r k_{2eff} T_{ref} {X_7}^2X_8} \quad \textbf{(6.26)}$$

$$\frac{\partial H_{AH}}{\partial X_8} = -\frac{T_g \ln\left(\frac{X_1 + X_2 + X_3 + X_4}{X_1}\right)(X_5 + X_6 + X_7)}{X_7 X_8{}^2 3.54\times10^{16} t_r k_{2eff} T_{ref}} \quad \textbf{(6 . 27)}$$

Overall, Equations 6.23 to 6.27 can be used to estimate the mean absolute humidity and its relevant statistics.

6.3 REPEATABILITY

An analysis of variance (ANOVA), which is also called an F-test, was used to test the repeatability of LDI2. The F-test enables comparison of the means of two or more independent sample populations containing mutually independent observations. The null hypothesis, H_0, can therefore be defined:

$$H_0 : \mu_1 = \mu_2 = \mu_3 = \ldots \mu_k \quad \textbf{(6 . 28)}$$

A one-way F-test will be used as the groups are only classified on one independent variable. The F test statistic is found by dividing the between group variance by the within group variance. The F-test statistic is thus defined (Miller, Freund, & Johnson, 1990):

$$F = \frac{n\sum_{i=1}^{k}\frac{\left(\bar{x}_i - \bar{\bar{x}}\right)^2}{k-1}}{\sum_{i=1}^{k}\sum_{j=1}^{n}\frac{\left(x_{ij} - \bar{x}_i\right)^2}{k(n-1)}} \quad \textbf{(6 . 29)}$$

Where:

F	=	F statistic
k	=	Number of populations
n	=	Number of sample points within each population
$\bar{x}$	=	Mean from the population
$\bar{\bar{x}}$	=	Overall mean of all populations

F is a random variable having the F distribution with $(k-1)$ and $k(n-1)$ degrees of freedom. The null hypothesis will be rejected when F exceeds the value of F evaluated at $(k-1)$ and $k(n-1)$ degrees of freedom. A p-value for the null hypothesis can be used. A p-value > 0.05 supports the null hypothesis that there is a 95% chance that $\mu_1 = \mu_2 = \mu_3 = \ldots \mu_k$.

Using the F-test requires the following assumptions about the population samples:

- all sample populations are normally distributed.
- all sample populations have equal variance.
- all observations are mutually independent.

The F-test is known to be robust to small violations of the first two assumptions (Miller, Freund, & Johnson, 1990).

The null hypothesis is that each cylinder measurement in time has equal means. If the F-test rejects the null hypothesis it is not possible to construct a pooled variance. A pooled variance of the raw measurements and mean concentrations will describe the precision and repeatability of the mean. Therefore, another method must be used to establish the precision and repeatability of compounds whose mean concentrations for each cylinder measurement in time are different.

The concentration measurement at each time step actually involves errors from both the precision and repeatability. The precision is how close values are to one another and can be represented as the variance. The repeatability is how close the average of the values is to the true value and can be represented as the bias. Often the true value is not known and therefore the bias cannot be estimated. This situation was the case in these repeatability tests, where the actual concentration of the tested compounds was not known. Therefore, the combined mean is defined as:

$$\bar{\bar{x}} = \sum_{i=1}^{k}\sum_{j=1}^{n} x_{ij} \qquad (6.30)$$

Where:

n = Number of sample points within each population

x = Sample point measurements within each population

$\bar{\bar{x}}$ = Overall mean of all populations

k = Number of groups (tested time steps)

The total variation about the combined mean can be defined as:

$$\begin{aligned} s_T^2 &= \frac{1}{nk}\sum_{i=1}^{k}\sum_{j=1}^{n}\left(x_{ij}-\bar{\bar{x}}\right)^2 \\ &= \frac{1}{k}\sum_{i=1}^{k}s_i^{\,2} + \frac{1}{k}\sum_{i=1}^{k}\left(\bar{x}_i-\bar{\bar{x}}\right)^2 \\ &= s_W^2 + s_B^2 \end{aligned} \qquad (6.31)$$

Where:

$\bar{x}$ = mean from the population

s_W^2 = within group variation

s_B^2 = between group variation

Note that s_T can be regarded as the total "standard deviation" about the combined mean, while s_W and s_B can be thought of as the within group variance and between group variance respectively. Note that s_W is a measure of the precision. However, s_B is not a measure of the bias, as the true concentration is not known and may well be different from the average concentration given by the machine.

6.4 DISPLAY OF RESULTS

The integrity of samples stored in Tedlar™ bags is to be described using the Cauchy distribution. It is important to display the results from the experiments to best indicate the

sample integrity as represented by Cauchy distributions in time. At every sample time, t_i, each of the three bags is sampled separately to give three concentrations for each monitored compound from each individual bag, as shown in Figure 6.3. In time, the concentrations in each bag move relative to one another which results in inter-bag variation.

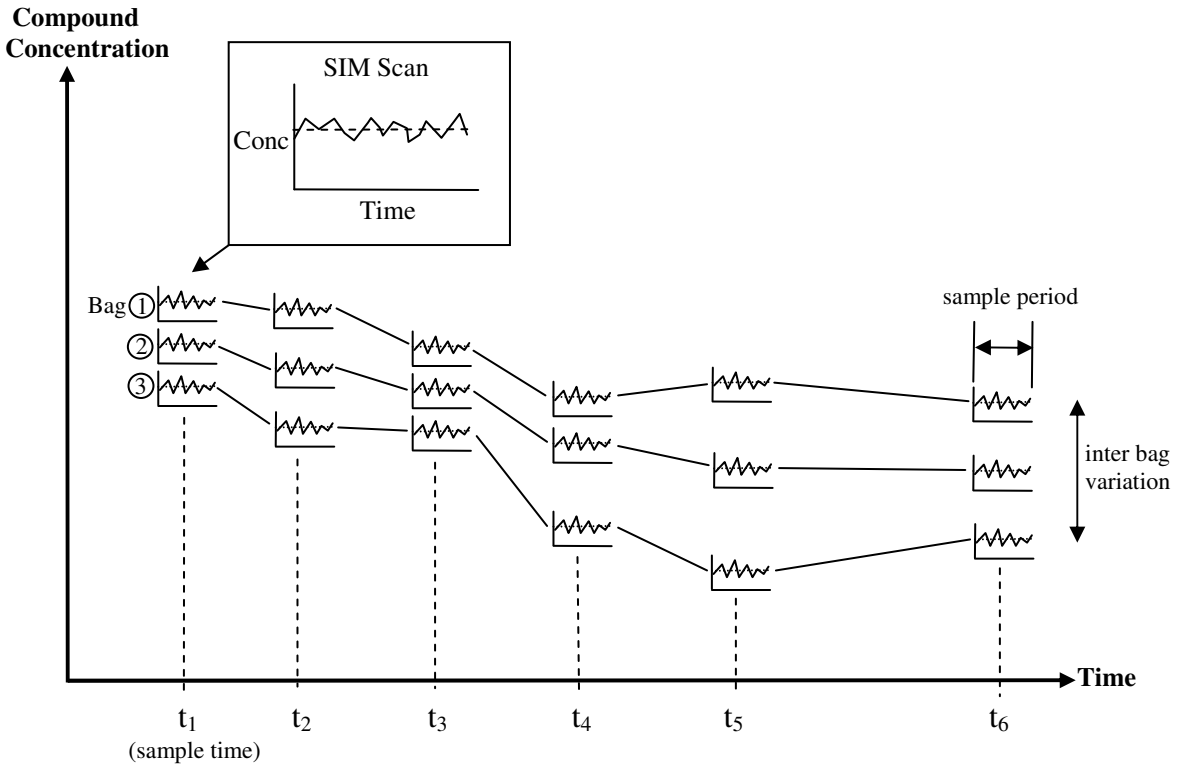

Figure 6.3: Individual SIM scans for each bag at the same sample time, indicating how they alter over the total test time to give a combined behaviour

The Cauchy distribution was used to combine the concentrations in each of the three bags to give an overall concentration distribution at each sample time, t_i. The shape of the distribution indicates the inter-bag variation, as shown in Figure 6.4. The location of the distribution peak is the combined mean while the distribution width and peak height

(density) indicate the variation and relative differences in the individual bag concentrations. Closer concentrations give a narrow distribution, sharper peak (higher density). As bag concentrations move apart from each other, a second peak occurs in the distribution. Therefore, a two peak distribution indicates that two bags have a similar concentration and the third differs. Three peaks indicate that each bag has a different concentration.

To view the combined Cauchy distributions for each sample time over the total testing time a contour plot was used. The contours indicate the density at each concentration within the range of the combined density. Therefore, a two dimensional graph of combined concentration versus time is made with contours of density. Because the distributions are localised at set sample times over the total testing time they appear as strips and therefore the plots are referred to as strip plots, as shown in Figure 6.5. Figure 6.5 shows the same three results as in Figure 6.4 and their transformation to a strip plot format to better aggregate the data presentation.

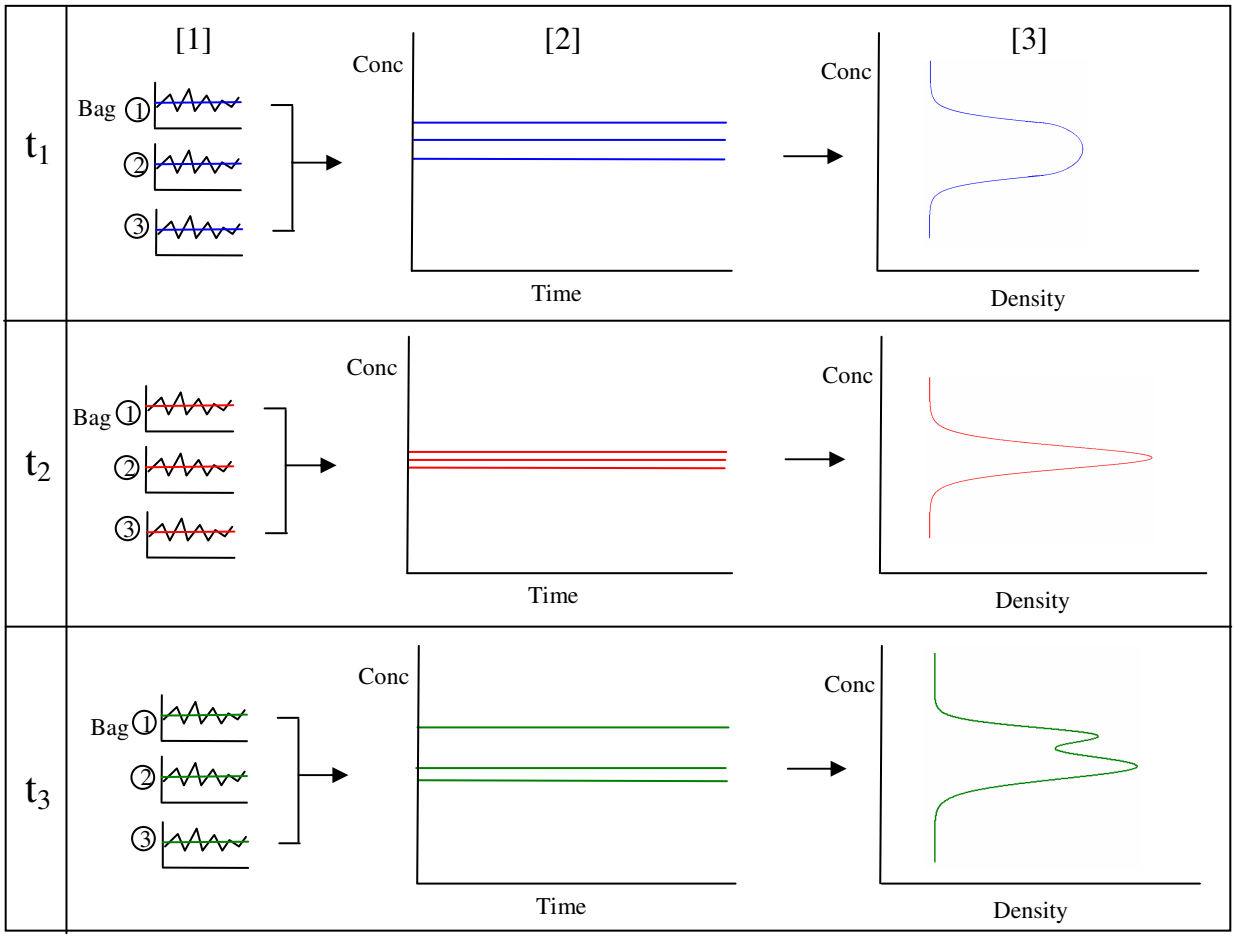

Figure 6.4: Combination of three individual bags to give a single combined Cauchy distribution at each sample time, where:

[1] the individual bag concentrations measured during sampling time t_i

[2] individual bag concentration during the sampling time t_i plotted on the same axis

[3] individual bag concentration combined to give a collective concentration Cauchy distribution of all three bags at sampling time t_i

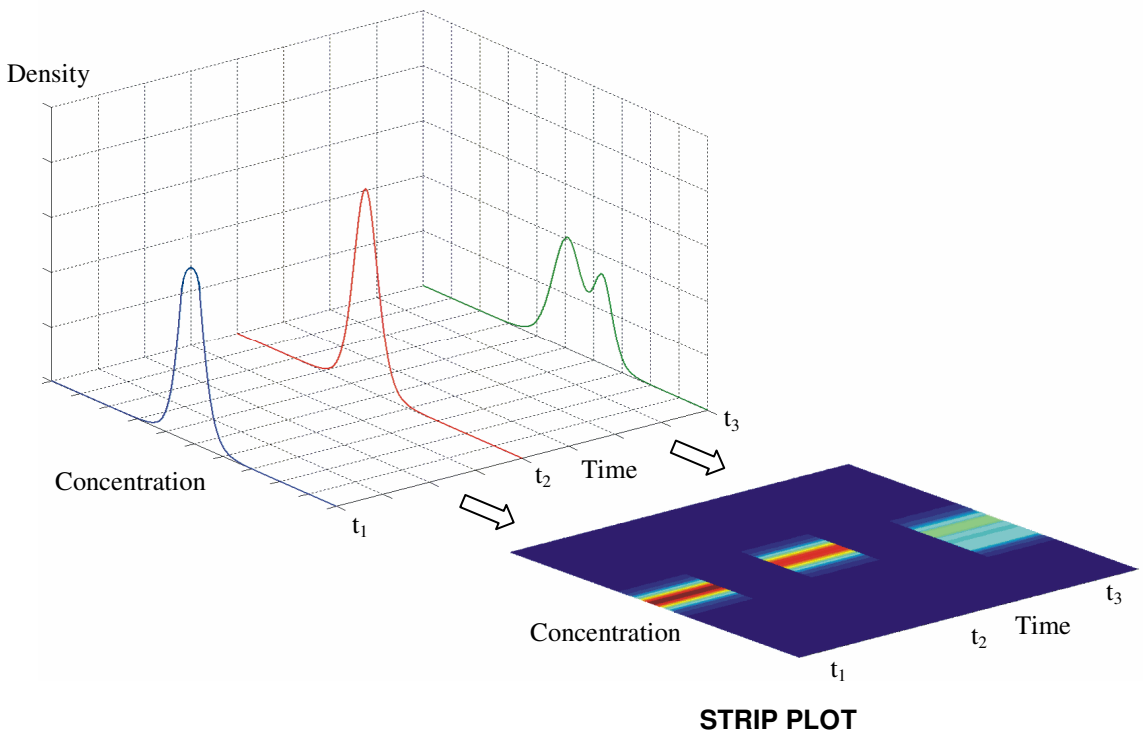

Figure 6.5: Cauchy distribution for the combination of three bags and its representation as a strip plot

It is possible to obtain an overall combined mean and two standard deviation variance from the Cauchy distribution and plot these as single points instead of a contour plot, as shown in Figure 6.6. Plotting the combined mean and variation enables combination of multiple storage conditions in one plot, which makes comparisons easier and reduces the number of plots. However, the overall combined mean and variation are not an accurate or necessarily useful indication of the inter-bag variation and incorrect conclusions can result. In Figure 6.6, it is clear from the strip plot that the bags begin at the same concentration and move apart over time to give three separate mean concentrations. This result is not obvious when looking at the overall combined mean and variance plot below

in Figure 6.6. It should be noted that the mean and variance plot does show the gradual and significant widening. However, it cannot indicate the separation into three distinct peaks, as the strip plot shows, even though it might be inferred by experience. Therefore the strip plot provides additional information about the inter-bag variation, which is useful in drawing conclusions on the bag behaviour.

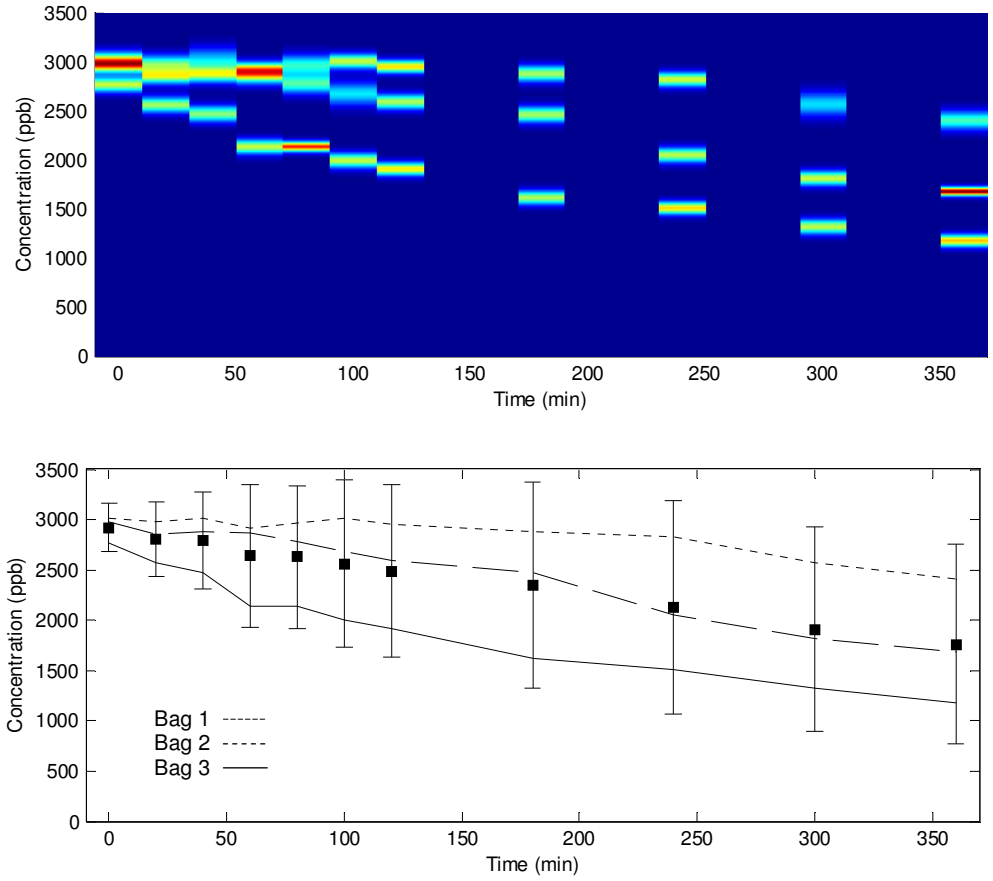

Figure 6.6: Contour plot (top) and the equivalent combined mean and variation (bottom) plot including the concentration for each bag

To include a measure of the inter-bag variation for the mean and two standard deviation variance plot, each bag concentration can be plotted as shown in Figure 6.6 and 6.7. Comparing the strip plot and each bag concentration indicates the effectiveness of the

Cauchy distribution. When two bag concentrations are close to each other, as at time 60 min in Figure 6.7, the Cauchy distribution gives a single peak with a high density. The third and lower peak at time 60 min is the third bag which differs from the other two. As the two bags which were initially together at time 60 min move apart at time 80 min, the single peak becomes two, giving a total of three peaks at time 80 min, as noted by the separate green bars in the top set. This situation represents three separated concentrations in each bag. It can therefore be seen that the concentration location of the Cauchy peaks align well with the bag concentrations. The Cauchy distribution also includes the precision of the measurements, which can be seen from looking at bag three. The reason for the high peak of bag three at time 80 min as compared to times 60 min and 100 min is that there was a higher precision in the measurements taken at time 80 min.

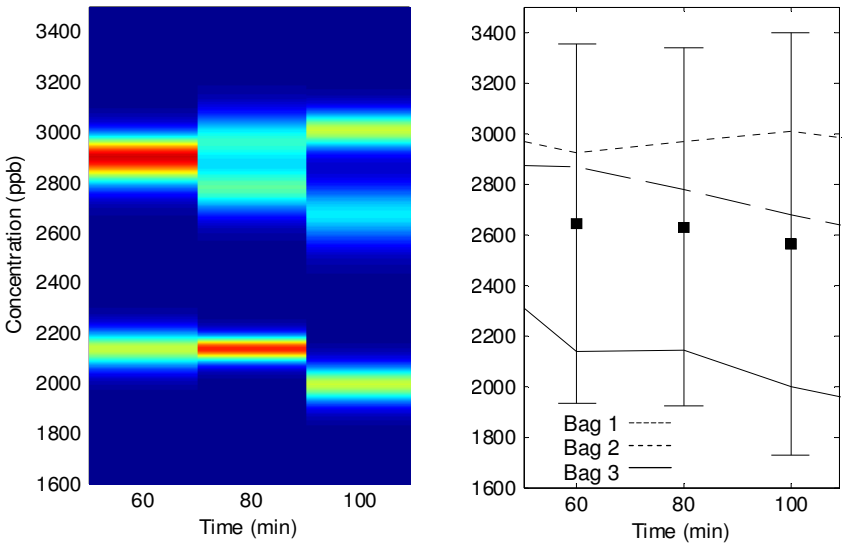

Figure 6.7: Comparison of the strip plot and each bag concentration in time

6.5 ANALYSIS SOFTWARE

Due to the large volume of data produced during experimentation and subsequent data-mining for analysis, software was written to automate these processes. Using software to calculate all aspects of the analysis, such as compound concentrations, was important, especially when optimising the kinetics and repeating the analysis to incorporate changes. Software also facilitated quick results from any re-tests or new compounds that were analysed. Automation software would also be mandatory if large scale experiments were carried out based on numerous bags (≈ 100), which is necessary to give a better idea of the statistical behaviour of the integrity of samples stored in Tedlar™ bags.

The software was written using Visual Basic and Matlab. Visual Basic was required as LDI2 outputs the scan as an Excel CSV file. The CSV file contained all aspects of the analysis including the counts for all monitored masses and calculated compound concentrations. It also contains important machine settings, such as flow tube temperature, primary and secondary gas flows.

Although the CSV file contained the calculated concentrations, they were not used and were instead re-calculated to enable complete control over the analysis. Visual basic code was thus written to select chosen precursor and product masses, and apply chosen rate coefficients and branching ratios to calculate the compound concentrations. The code also selected the masses used to calculate the humidity and pick out important aspects required in the calculation, such as the flow tube temperature.

Once the Visual basic code was run on each CSV file, it was saved as a normal Excel file according to the bag number and analysis time. Matlab was then used to open each saved Excel file, obtain the required data, and carry out analysis including the humidity calculation and the statistical analysis to produce the strip plots for both humidity and compound concentration.

The Visual Basic and Matlab code that was written enabled:

- Calculation of the humidity and concentration for ammonia, isoprene, ethanol, pentane and acetone from a given Excel CSV scan file.
- Re-calibration using any given reaction rate coefficient and choice of which product masses or precursor counts to omit or include.
- Choice of the number of points to analyse within each sample period
- Choice of the number of bags to be combined
- Filtering to remove outliers given a specified spike filter level
- Calculation of statistics including averaging, variance and the combined Cauchy distribution for each compound concentration and humidity for number of bags.
- Plotting of the results including the Cauchy distribution strip plots and overall combined mean and two standard deviation variance from the Cauchy distribution as single points.

This code thus enables a complete end-to-end analysis as described in this chapter.

6.6 SUMMARY

The analysis developed involved the kinetics analysis, statistical analysis, and the development of specialised software written to carry out the actual analysis.

The kinetics involved the choice of reaction rate coefficients, branching ratios and monitored precursor and product masses, all of which were optimised. A generalised Cauchy distribution was used to give a combined distribution for the humidity and compound concentration, including a mean and variance and the inter-bag variation. An F-test was used to test the repeatability of LDI2 and a total "standard deviation" about the combined mean, s_T, was used as a measure of the combined effect of the precision and repeatability.

To view the combined Cauchy distributions for each sample time over the total testing time a contour plot of the distribution density was used and was termed a strip plot. Due to the large volume of data produced during experimentation and subsequent data-mining for analysis, software was written to automate these processes.

This chapter described the analysis methods used to best indicate the integrity of samples stored in Tedlar™ Bags and the machine repeatability and precision. The next chapter will look at the results from the experiments used to test the machine repeatability and precision.

6.7 REFERENCES

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7 MACHINE REPEATABILITY & PRECISION

The machine repeatability and precision were established by testing a cylinder containing constant amounts of acetone, ethanol and pentane over time. By looking at the equations used to calculate the compound concentration, it was possible to outline the factors affecting the repeatability and precision and obtain a measure of their relative contributions.

Studies reported a precision of better than 10% over a range of 10 parts per billion (ppb) to 20 parts per million (ppm) for SIFT-MS (P Spanel, Cocker, Rajan, Smith, & 1997; Smith et al., 1998). However, it is not acceptable to only consider the precision when analysing samples in time. The repeatability is also important and has particular importance in a clinical applications

As discussed in chapter 6, the best measurement of the precision and repeatability is a pooled variance and mean, which is only applicable if the null hypothesis for the F-test was accepted. The null hypothesis was that each cylinder measurement in time had equal means. A p-value > 0.05 was used to accept or reject the null hypothesis. If

the means are not equal then the s_T value from Equation 6.31 can be used as a measure of the precision and repeatability.

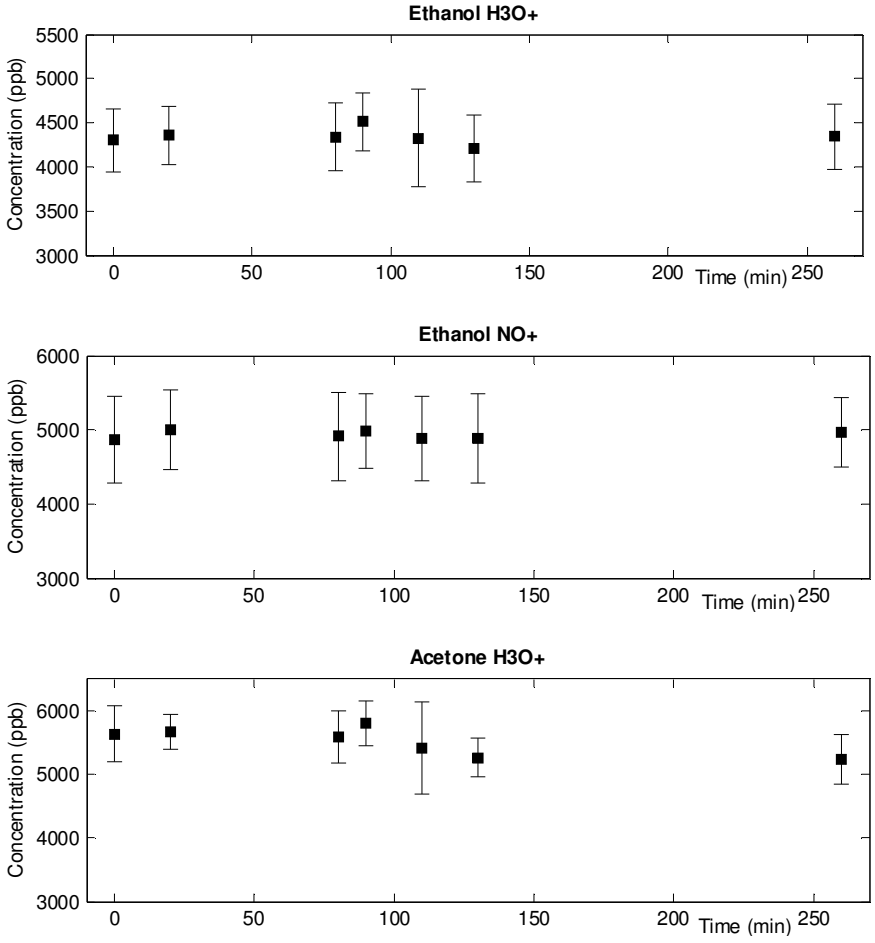
All compound measurements had p-values less than 0.05, except for pentane using the O_2^+ precursor and ethanol using the NO^+ precursor as shown in Table 7.1. Therefore, in most cases, the cylinder measurements did not have equal means in time. Thus, a pooled variance and precision could not be estimated, and the s_T value was the best approach. The s_T values ranged between 217 – 349 ppb as shown in Table 7.1.

Compound	Precursor	Average Conc (ppb)	P-Value	s_W (± ppb)	s_B (± ppb)	s_T (± ppb)
Pentane	O_2^+	2172	0.97	347	30	349
Ethanol	H_3O^+	4340	8.0E-05	223	201	300
	NO^+	4930	0.42	261	174	314
Acetone	H_3O^+	5515	0	200	84	217
	NO^+	4106	4.0E-12	282	52	287

Table 7.1: Summary of repeatability and precision for tested compounds

However, the s_T value involve contributions from both the within group variance, s_W, and between group variance, s_B. For pentane, the low s_B indicates good repeatability, while the high s_W shows low precision which can also been seen in Figure 7.1. The combination of the low s_B and high s_W gave it the largest s_T value, but the large s_T value does not indicate it is the worse measurement. For measurements of the bags in time, the repeatability is more important than the precision because it is possible to average the variances and obtain a better estimate, while averaging the between group variance does not improve the result. Therefore, having a small s_B, thus good repeatability, is most important.

It can be seen from Figure 7.1 that the concentrations for both acetone and ethanol do not agree between the H_3O^+ and NO^+ precursors. The disagreement is due to the kinetics used. It was possible, using the software developed, to re-calibrate the rate coefficients used in the kinetics so that the concentrations given using the H_3O^+ and NO^+ precursors agreed. However, the rate coefficients affect the variance in the concentration. Therefore, to ensure the precision and repeatability is applicable to the kinetics used in the bag testing, the same rate coefficients were used.



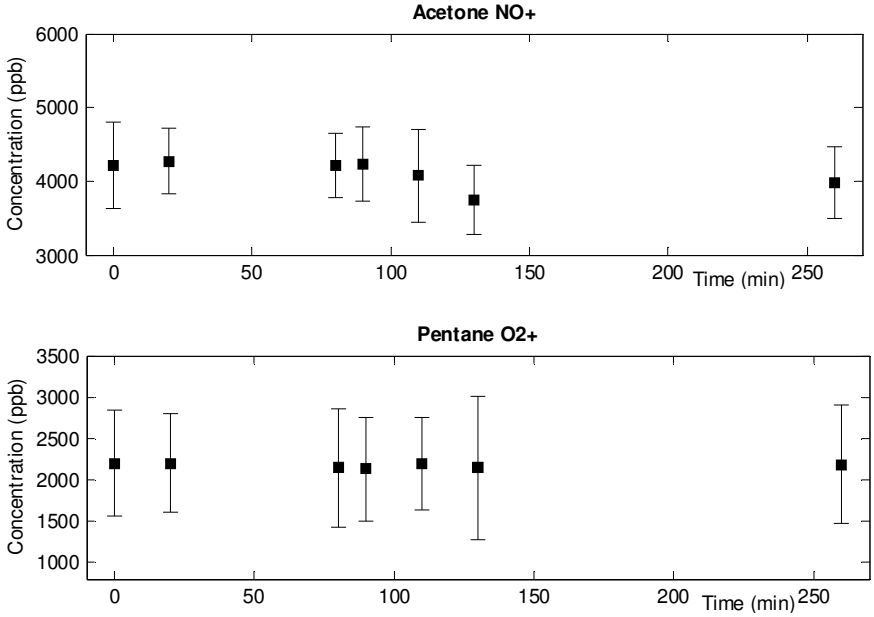


Figure 7.1: Mean compound concentration for relevant precursor over time including 95% confidence interval

7.1 FACTORS AFFECTING THE REPEATABILITY & PRECISION

The factors affecting the repeatability and precision of the concentration measurements can be divided into machine dependent settings and compound dependent settings. Equation 2.17 is used to calculate the compound concentrations can be written explicitly in terms of the machine and compound dependent measurements and thus defined:

$$[A]_{ppb} = \frac{T_g^{\,2}\Phi_{Total}^2}{p_g^{\,2}} \frac{1}{\Phi_S} \frac{R\times\left(1\times10^{12}\right)\times1.5\times4}{N_a(l-\varepsilon)\pi d_t^{\,2}T_{ref}} [B] \qquad (7.1)$$

Where:

Φ_{Total} = total flow in flow tube (torr Ls^{-1})

Φ_S = sample flow through capillary (torr Ls^{-1})

T_g = flow tube gas temperature (K)

p_g = flow tube pressure (torr)

d_t = flow tube diameter (m)

T_{ref} = reference temperature for rate coefficients (273 K)

l = reaction length (m)

ε = end correction

R = gas constant (62.4 $LtorrK^{-1}mol^{-1}$)

N_a = Avogadro's number ($6.022\times10^{23}\,mol^{-1}$)

$$[B] = \frac{\sum_{i=1}^{n} \frac{I_{si}D_{fi} + I_{\sec i}D_{fi}}{b_{ri}}}{\sum_{i=1}^{m} k_i I_{pi} D_{fi}}$$

I_{pi} = precursor ion counts for mass i (cps)

I_{si} = primary product counts for mass i (cps)

$I_{\sec i}$ = secondary product ion counts for mass i (cps)

k_i = rate coefficient (molecules $cm^3\ s^{-1}$) for mass i

D_{fi} = combined differential diffusion & mass discrimination factor for mass i

Taking out the factors that remain constant from Equation 7.1 and combining them into one constant, C, gives:

$$[A]_{ppb} = C\ \frac{T_g^{\,2}\Phi_{Total}^2}{p_g^{\,2}}\ [B] \qquad (7.2)$$

Therefore, the precision and repeatability of the concentration measurements, $[A]_{ppb}$, arise from the precision and repeatability in T_g, Φ_{Total}, p_g and $[B]$. The values of T_g, Φ_{Total}, p_g are all physically measured, machine specific quantities and are not dependent on the compound analysed. As T_g, Φ_{Total}, p_g are squared, the effect of their individual precision on the total precision of $[A]_{ppb}$ is increased. As [B] includes measurements of the compound masses, it is compound and precursor dependent, and is affected by factors such as differential diffusion and mass discrimination. However, $[B]$ also includes some machine specific quantities associated with the detector used to measure the count rates.

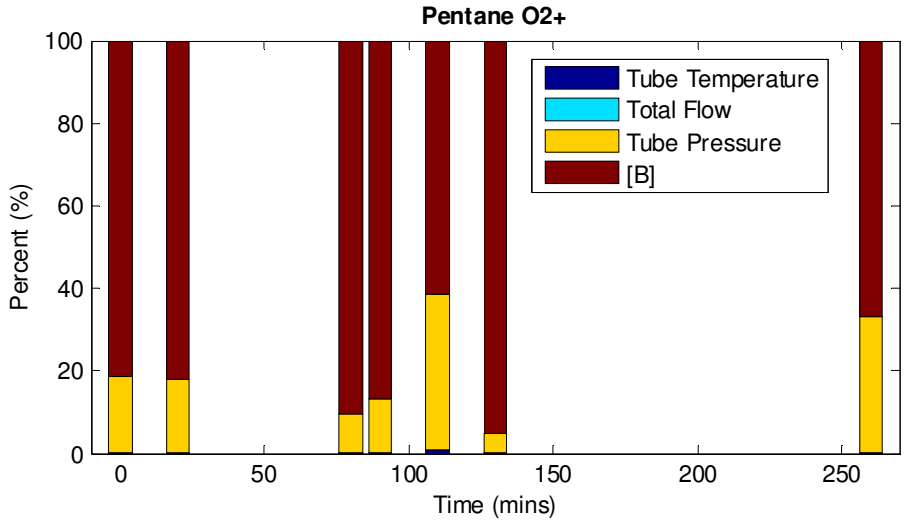
Plotting the average and 95% confidence intervals for T_g, Φ_{Total}, p_g and $[B]$ gives an indication of the repeatability and precision of each of these measurements for each compound and precursor combination. This information is provided in Appendix A4. More generally, it is possible to measure the significance of the contribution of T_g, Φ_{Total}, p_g and $[B]$ to the precision of $[A]_{ppb}$, while the contribution to repeatability is more difficult.

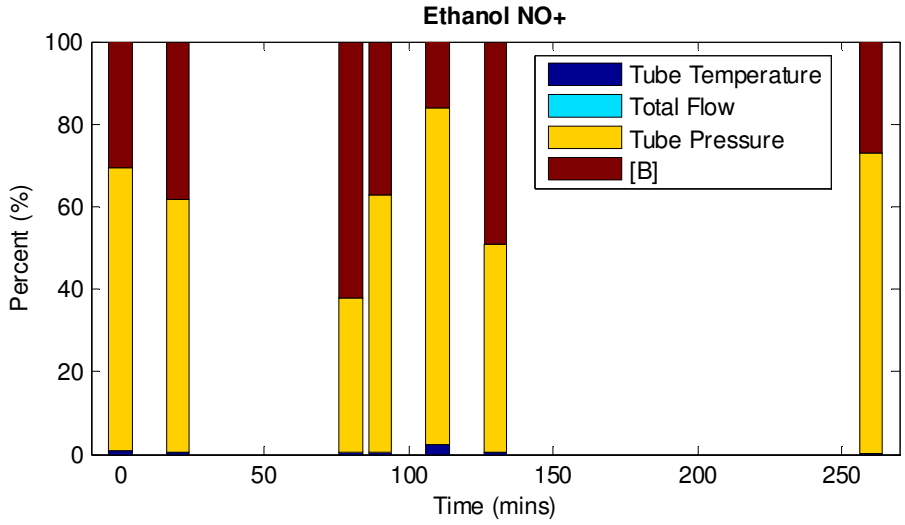
The contribution to the overall precision in $[A]_{ppb}$ from T_g, Φ_{Total}, p_g and $[B]$ can be found by looking at the contribution of each quantity to the total variance of $[A]_{ppb}$. As with the humidity, using the delta method, a first order multivariate Taylor expansion can be used to linearise $[A]_{ppb}$ with respect to each measured quantity. Therefore:

$$Var([A]_{ppb}) \approx \left(\frac{\partial [A]_{ppb}}{\partial T_g}\right)^2 Var(T_g) + \left(\frac{\partial [A]_{ppb}}{\partial \Phi_{Total}}\right)^2 Var(\Phi_{Total}) + \left(\frac{\partial [A]_{ppb}}{\partial p_g}\right)^2 Var(p_g) + \left(\frac{\partial [A]_{ppb}}{\partial [B]}\right)^2 Var([B]) \quad (7.3)$$

Comparing the contributions from each measured quantity to the overall variance indicates the significance, as seen in Figure 7.2. The results indicate the main contributors to the precision of $[A]_{ppb}$ are p_g and [B], indicating that the precision is both machine and compound related. More specifically, both quantities account for at least 95% of the precision.

However, the magnitude of the contribution of all factors to the precision of $[A]_{ppb}$ differs in time, with different precursor and compound concentrations, and no obvious trends can be seen. The contribution to the precision of pentane measured by the O2+ precursor from [B] was much more significant than the contribution from p_g. In general, $[A]_{ppb}$ using the H3O+ precursor had higher percentage contributions from p_g than [B] when compared to the NO+ precursor, which was true for both acetone and ethanol as well. This result was surprising as the H3O+ precursor involved the monitoring of 4 precursor related masses compared to the 2 masses for NO+ (Table 6.2 and Appendix A5). However, as the samples were dry, there would have only been significant contributions from the 19 H3O+ precursor mass and 30 NO+ precursor mass and thus both the NO+ and H3O+ precursors would have had one significant monitored precursor mass. This situation would have also been true for the products where all water clusters would be insignificant, as the samples were dry.


Pentane O2+
Tube Temperature
Total Flow
Tube Pressure
[B]
Percent (%)
Time (mins)
0
20
40
60
80
100
0
50
100
150
200
250


Ethanol NO+
Tube Temperature
Total Flow
Tube Pressure
[B]
Percent (%)
Time (mins)
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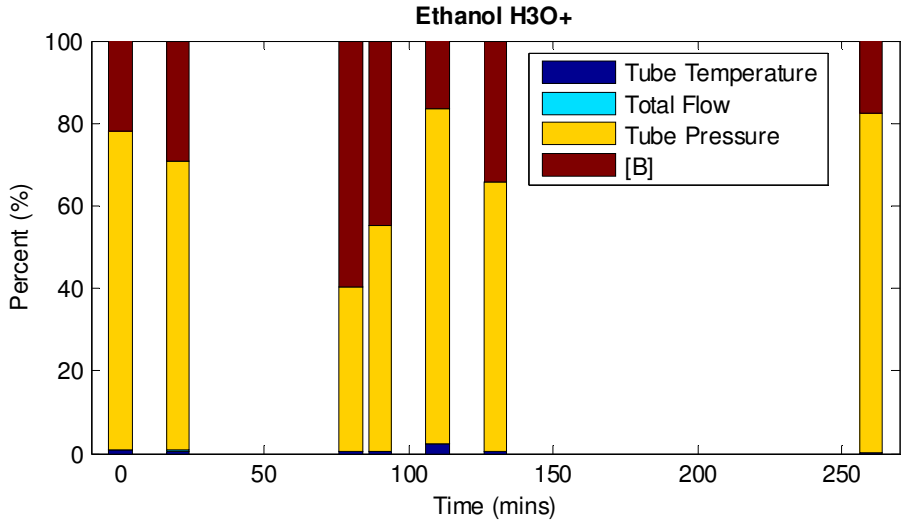
20
40
60
80
100
0
50
100
150
200
250


Ethanol H3O+
Tube Temperature
Total Flow
Tube Pressure
[B]
Percent (%)
Time (mins)
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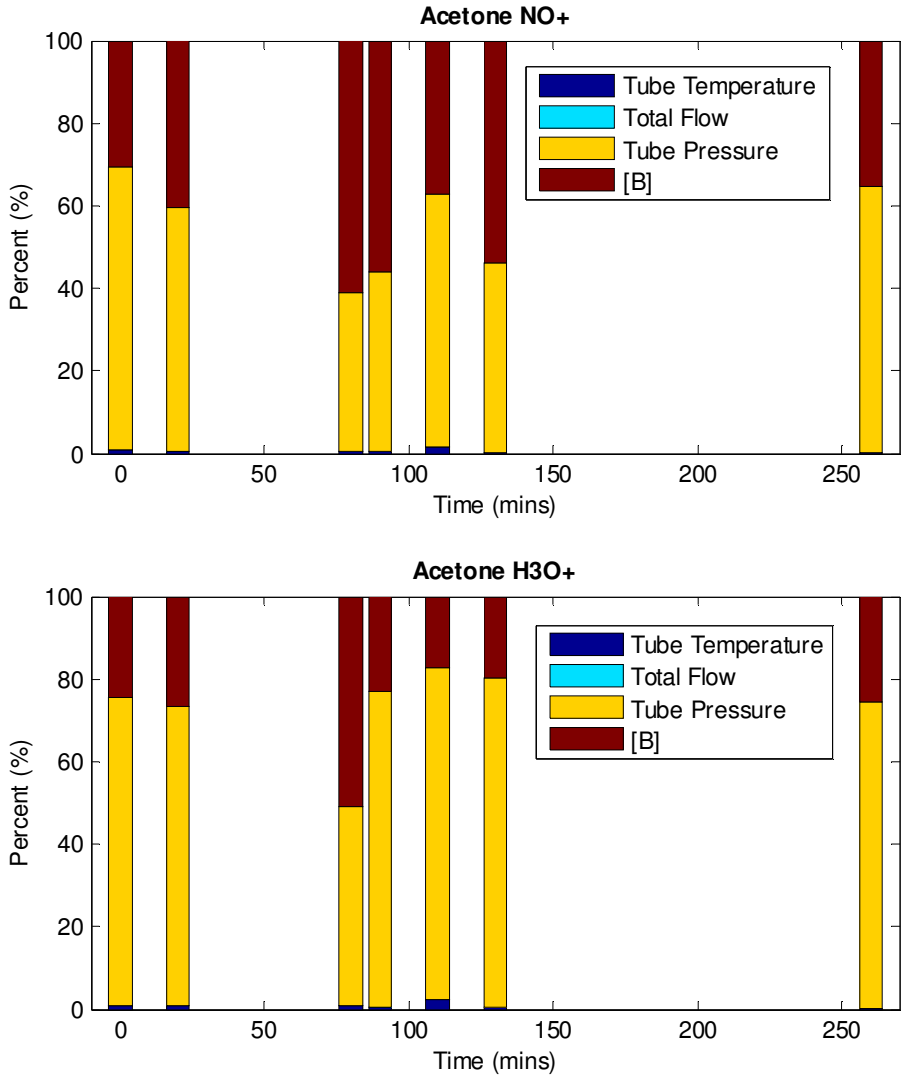


Figure 7.2: Percent contribution of tube temperature (T_g), total flow (Φ_{Total}), tube pressure (p_g) and [B] to the total precision in $[A]_{ppb}$ for each compound and precursor in time.

It is difficult to obtain a good estimate of the contribution from T_g, Φ_{Total}, p_g and [B] to the repeatability of $[A]_{ppb}$. The difficulties arise because it is not possible to combine the s_B in a linear fashion, as was done for the variance. The best approach is to evaluate the repeatability of each T_g, Φ_{Total}, p_g and [B] by looking at the p-value from the F-test, as shown in Table 7.2. All components except for the [B] pentane O_2^+, suggested that the null hypothesis, that all the means were equal, should be rejected.

Therefore, all the measured quantities could be the source of the repeatability in $[A]_{ppb}$. However, the significance of their contribution is still unknown.

Calculation Component	Average Value	P-Value	s_W (±)	s_B (±)	s_T (±)
T_g	298.1 K	9.53E-05	0.67 K	0.27 K	0.73 K
p_g	0.6 torr	3.42E-06	0.0079 torr	0.0037 torr	0.0088 torr
Φ_{Total}	43.4 torrL/s	0	0.012 torrL/s	0.012 torrL/s	0.017 torrL/s
[B] Pentane O_2^+	9.4.E+06	0.33	8.4E+05	1.7E+05	8.5E+05
[B] Acetone H_3O^+	2.4.E+07	0	5.7E+05	1.2E+06	1.3E+06
[B] Acetone NO^+	1.8.E+07	0	6.1E+05	9.6E+05	1.1E+06
[B] Ethanol H_3O^+	1.9.E+07	0	4.8E+05	5.0E+05	7.0E+05
[B] Ethanol NO^+	2.1.E+07	3.2E-10	6.2E+05	3.8E+05	7.3E+05

Table 7.2: Repeatability of the factors associated with the calculation of $[A]_{ppb}$

It is also important to mention the contribution to the precision and repeatability of carrying out the experiments. Inconsistencies in carrying out the experiments would have some contribution to the repeatability and precision observed. However, these errors were minimised by using a dry (0.05% absolute humidity) sample, which was plumbed directly into the inlet of LDI2.

7.2 SUMMARY

The total "standard deviation" of the mean, s_T, values ranged between 217 – 349 ppb for ethanol, acetone and pentane. The factors affecting the repeatability and precision of the concentration measurements, $[A]_{ppb}$, were machine dependant (T_g, Φ_{Total}, p_g) and compound dependant ([B]). The main contributors to the precision of $[A]_{ppb}$ were p_g and [B]. The magnitude of the contribution of all factors to the precision $[A]_{ppb}$ differed in time, with different precursor and compound. All factors except for the [B]

pentane O_2^+ could be the cause of the poor repeatability in $[A]_{ppb}$ as well as experimental error.

This chapter described the established machine repeatability and precision. The next chapter will look at the results from the experiments used to test the sample integrity of acetone, isoprene, ethanol and pentane, ammonia including the effect of the bag storage size on the sample integrity.

8 SAMPLE INTEGRITY

The integrity of samples stored in Tedlar™ bags was tested using three experiments:

- effect of storage size
- sample integrity of acetone, isoprene, ethanol and pentane
- sample integrity of ammonia

The storage temperature, sample humidity, storage time, inter-bag variation, combined variance, precursor used, and reproducibility were studied to establish trends in the sample integrity. This chapter draws out the main results and trends from the plots located in the Appendices A1 to A3. The plots in Appendices A1 to A3 include all experiment runs, the second reproducibility runs and background permeation tests.

8.1 EFFECT OF STORAGE SIZE ON SAMPLE INTEGRITY

No obvious effects of storage size on the sample integrity of pentane, isoprene, ethanol and acetone were observed from the strip plots, as detailed in Appendix A2. All compounds behaved in a similar manner and had comparable inter-bag and intra-bag variation regardless of the bag size as shown in Figure 8.1.

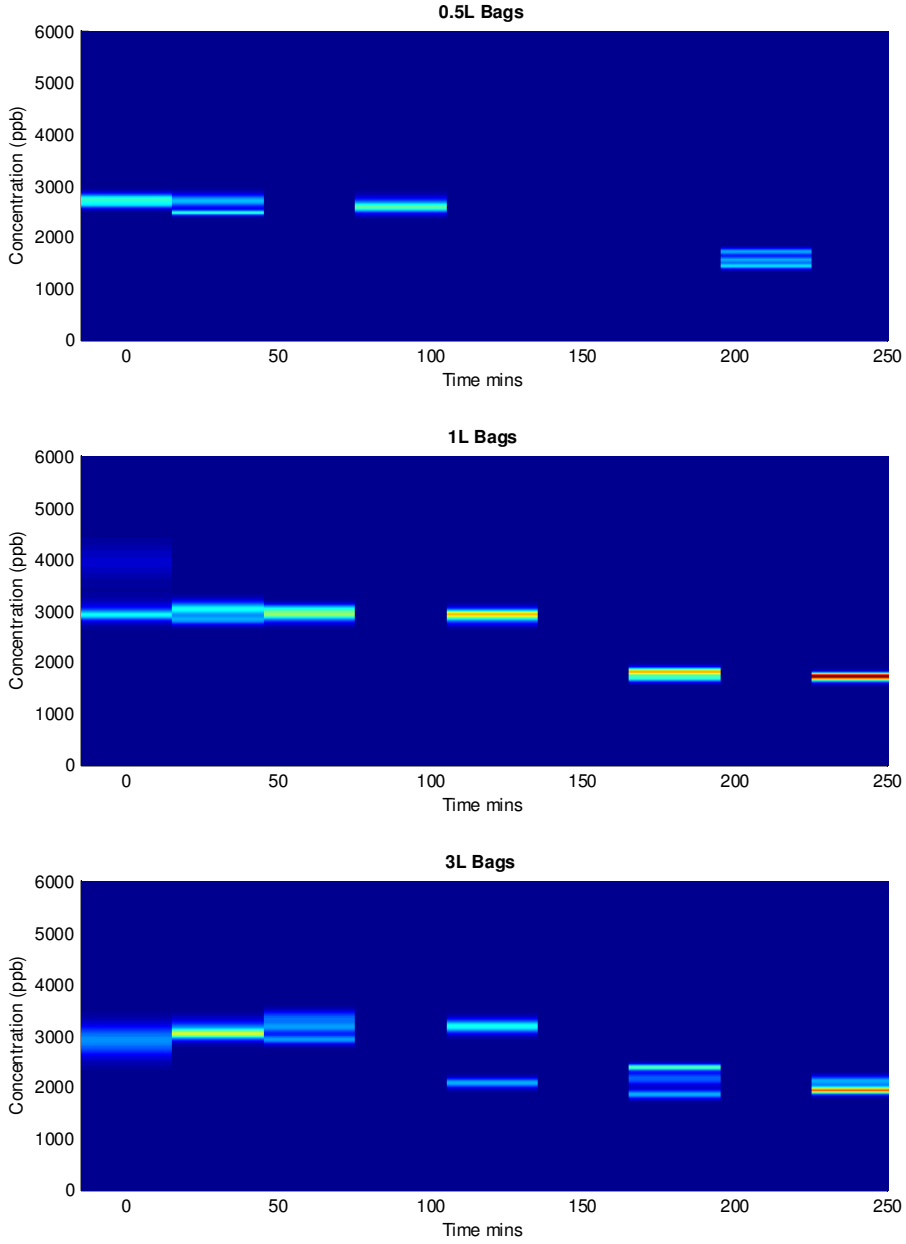


Figure 8.1: Ethanol monitored using the H3O+ precursor in 23°C-25°C stored breath for 0.5, 1 and 3 L Tedlar™ bags

To investigate the independence of sample integrity from the storage size further, the change in absolute humidity within the bags was observed as, shown in Figure 8.3 and 8.4. As water molecules are smaller than pentane, isoprene, ethanol and acetone, they are more likely to be affected by the factors associated with the surface area to volume

ratio and also bond strongly to surfaces which are slightly hydrophilic. These factors include permeation and condensation.

Each sample time, a volume of 0.048L was removed from each bag. The bags also had different surface areas in contact with the sample due to their different physical sizes. Therefore, the volume to surface area ratio changes over time and is different for each bag size, as seen in Figure 8.2. The change in percent absolute humidity within the bags, as calculated using Equation 2.26, was plotted along with the change in the volume to surface area ratio as samples were removed from the bag, as shown in Figure 8.3 and 8.4.

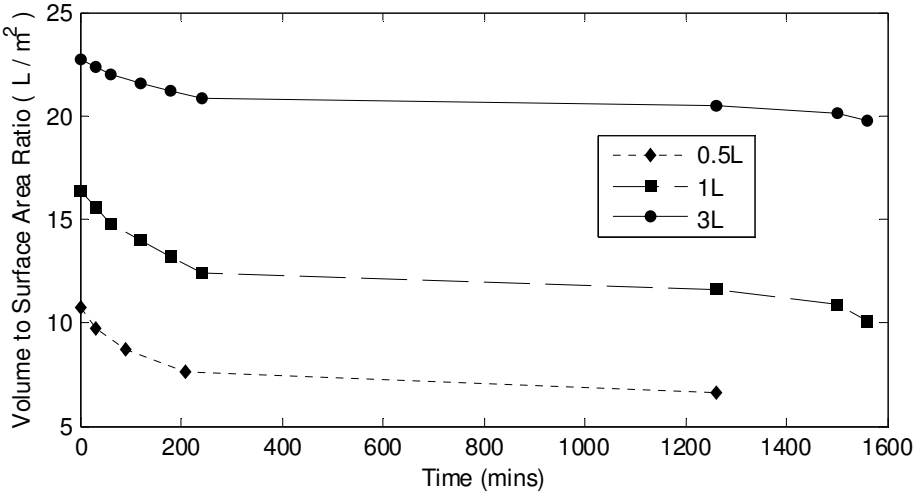


Figure 8.2: Volume to surface area with time for 0.5,1 and 3L Tedlar™ bags and a capillary sample rate of 2.4mLs^{-1} and sample time of 20 seconds

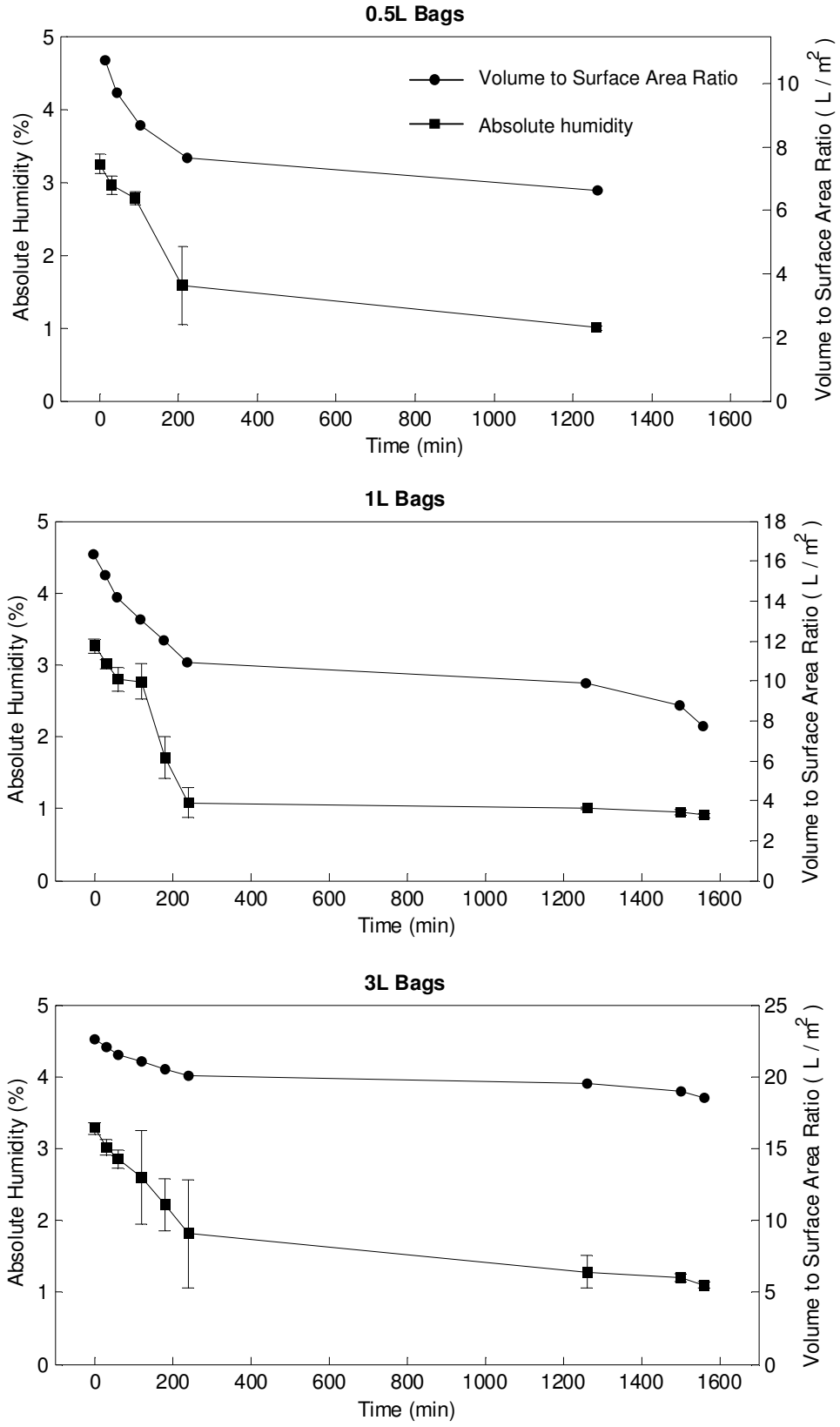


Figure 8.3: Combined three bag mean and 1.96 standard deviation of the absolute humidity as well as the volume to surface area ratio for 0.5,1 and 3 L, 23°C-25°C stored Tedlar™ bags

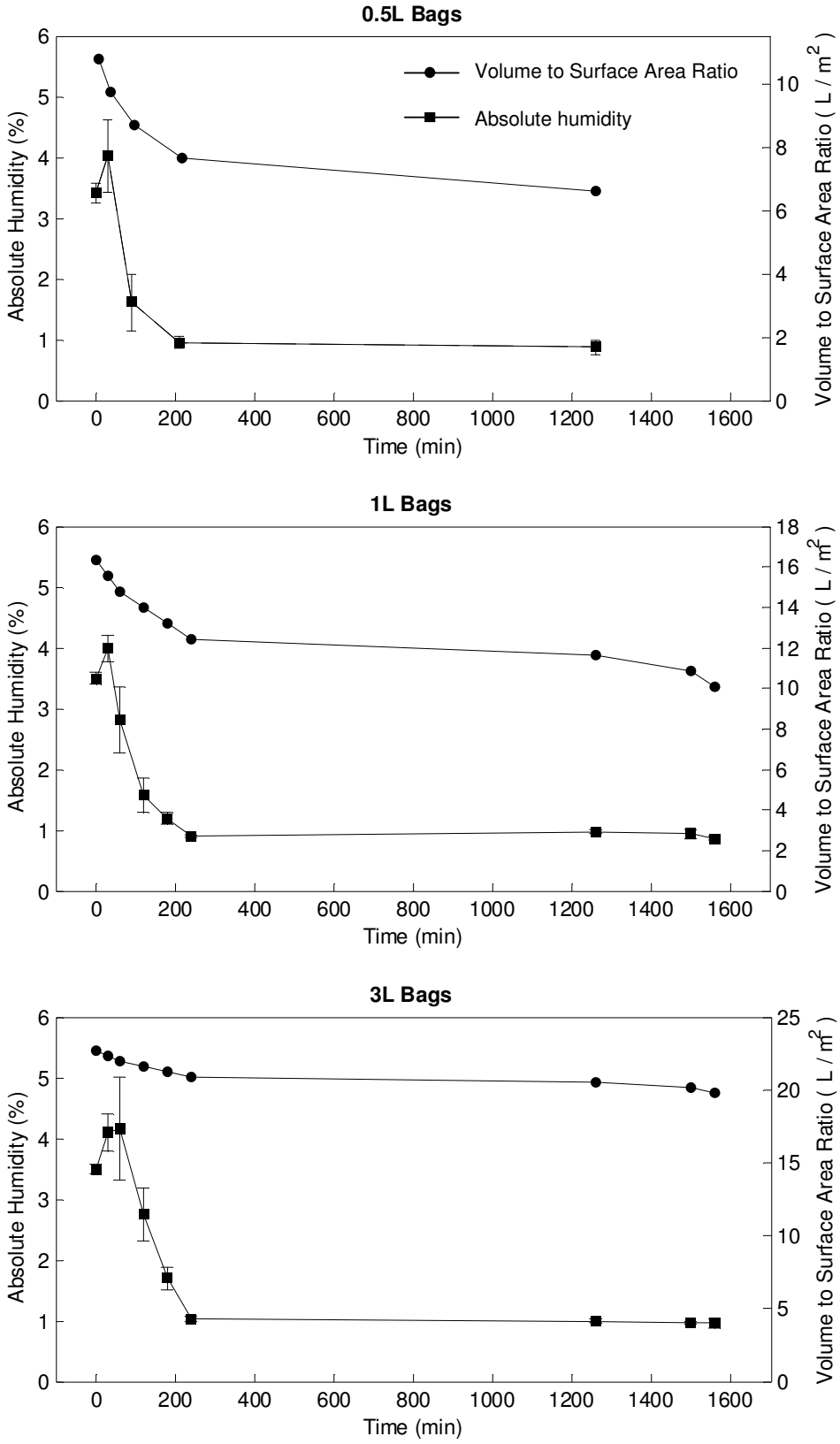


Figure 8.4: Combined three bag mean and 1.96 standard deviation of the absolute humidity as well as volume to surface area ratio for 0.5,1 and 3 L, 37°C stored Tedlar™ bags

Comparing the absolute humidity changes for the different bag sizes and storage conditions indicates that each bag has approximately the same starting and final mean absolute humidity of 3% and 1% respectively. All mean absolute humidity values between the bag sizes agree to within their combined 1.96 standard deviation limits. The combined 1.96 standard deviations of the mean absolute humidities are also comparable at each sample time for all three bag sizes and storage conditions. The combined 1.96 standard deviation is initially small but increases during the 60 to 240 min time period, then falls again.

An initial rise in the absolute humidity of the 37°C stored bags from time 0 to 20 min was observed for all bag sizes. The rise was due to the experimental method used. Bags were tested after being spiked and filled with breath exhalations, but before being put in the incubator. Therefore, the bags tested at time 20 min had been heated and thus contained more water in its vapour phase. The increase in bag absolute humidity with heating indicates the readiness of the water to condense on the bag surfaces. In the 37°C stored bags, the decay of absolute humidity occurs at similar rates. For the 23°C-25°C stored bags, the decay is faster for the 1L and 0.5 L bags compared to the 3L bags.

The trends in the volume to surface area ratio and the mean absolute humidity in Figures 8.3 and 8.4 for all bag sizes show a striking similarity. The similarity indicates that the changing volume to surface area ratio is responsible for the behaviour of the absolute humidity. However, this is more evident in the 23°C-25°C stored bags than the 37°C stored bags. In the 23°C-25°C stored bags, the rate of decay in the volume to surface area ratio of the 3L bags is smaller than in the 1L and 0.5L bags, which

corresponds to the smaller rate of decay in the absolute humidity for the 3L bags compared to the 1L and 0.5L bags.

The magnitude of the volume to surface area ratio does not seem to have a great effect, but rather the trend in the values. This is seen in the 23°C-25°C stored 0.5L, 1L and 3L bags where the absolute humidities at time 240 min are 1.6%, 1% and 1.8% respectively, while the values for the volume to surface area ratio are 7, 10 and 20 respectively. For the 37°C stored bags, the decay of the absolute humidity occurs almost identically between the three bag sizes, irrespective of the volume to surface area ratio. This result indicates that perhaps condensation is more problematic than permeation. The argument that condensation is more problematic than permeation arises because condensation would be more likely than permeation to occur in the 23°C-25°C stored bags, as less water would be present in the vapour phase. As the decay in absolute humidity for the 37°C stored bags is identical for all three bag sizes, permeation must not be significant. In comparison, in the 23°C-25°C stored bags, where condensation is more likely, and permeation less likely, the decay of the absolute humidity follows the volume to surface area ratio closely.

8.2 SAMPLE INTEGRITY OF ETHANOL, ISOPRENE, PENTANE & ACETONE

Each strip plot conveyed numerous aspects of the compound's behaviour, which included the background permeation, storage temperature and sample humidity effect, storage time, inter-bag variation and combined variance, precursor used and reproducibility. These aspects can be studied to establish trends in the sample integrity and provide possible explanations for the behaviour observed. This section draws out the main results and trends from plots located in Appendix A1.

8.2.1 BACKGROUND PERMEATION

Background permeation tests for isoprene, ethanol, acetone and pentane, as shown in Figure 8.5 indicated that the behaviour of the compounds was unlikely to be due to permeation of compounds from the atmosphere into the bags. Levels of compounds from time 0 to 360 min only changed by a maximum of 180 ppb, which occurred for acetone (NO^+) in the 37°C stored bags.

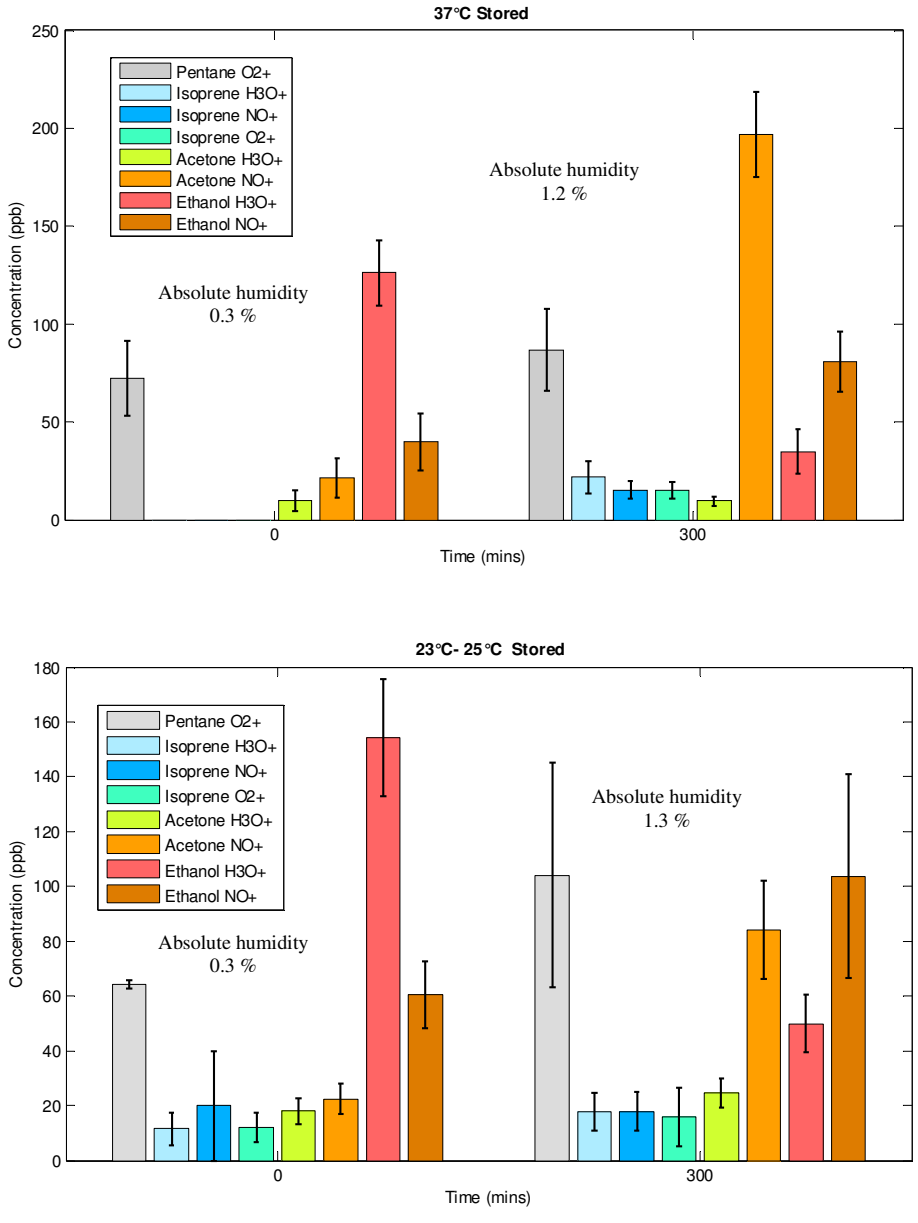


Figure 8.5: Nitrogen filled permeation Tedlar™ bag tested at time 0 and 360 min for both storage temperatures including the concentrations and 95% confidence interval

8.2.2 STORAGE TEMPERATURE & ABSOLUTE HUMIDITY

No compound in the bags filled with nitrogen displayed different behaviour for either storage temperatures as shown in Table 8.1. However, there were differences in behaviour between the storage temperatures in the breath substrate for ethanol (NO^+, H_3O^+) and acetone (NO^+). In the 37°C stored bags, acetone (NO^+, H_3O^+) and ethanol (NO+, H3O+) had higher starting concentrations compared to the 23°C-25°C stored bags. Isoprene (O_2^+, H_3O^+, NO^+) and pentane (O_2^+) had similar starting concentrations between both temperatures.

Different behaviour between the nitrogen-filled and breath-filled bags was displayed by ethanol (H_3O^+, NO^+) and acetone (H_3O^+), as shown in Table 8.1. For acetone (H_3O^+, NO^+) the breath substrate for both temperatures had higher starting concentrations, as shown in Table 8.2. Ethanol (H_3O^+,NO^+) had higher starting concentrations in the 23°C-25°C stored breath, along with ethanol (H_3O^+) in the 37°C stored breath. Ethanol (NO^+) had lower starting concentrations in the 37°C stored breath compared to the nitrogen. Isoprene (O_2^+, H_3O^+) had higher starting concentrations in the 37°C stored breath than the 37°C stored nitrogen. Isoprene (NO^+) had similar starting concentrations between both substrates. Pentane (O_2^+) had comparable starting concentrations between substrates.

Compound	Precursor	Different Concentration Behaviour for Breath & Nitrogen Filled bags	Different Concentration Behaviour for Storage Temperature	
			Nitrogen Filled Bags	Breath Filled Bags
Acetone	H_3O^+	✓	x	x
	NO^+	x	x	✓
Ethanol	NO^+	✓	x	✓
	H_3O^+	✓	x	✓
Isoprene	NO^+	x	x	x
	O_2^+	x	x	x
	H_3O^+	x	x	x
Pentane	O_2^+	✓	x	✓

Table 8.1: Effect of substrate and temperature on the behaviour of compounds and the relevant precursor where ✓ indicates an effect and x no effect

Compound & Precursor	Quantity	Nitrogen Filled Bags 37℃	Nitrogen Filled Bags 23℃- 25℃	Breath Filled Bags 37℃	Breath Filled Bags 23℃- 25℃
Acetone	Affected by humidity	≡	≡	x	x
H_3O^+	Time 0 min Levels (ppb)	2350	2000 - 2800	3700	2400 - 3600
	Time 360 min Levels (ppb)	1150 - 1250	1100 - 1200	2950 - 3150	2700 - 3050
	Change in concentration (ppb)	⇩1200 - ⇩1300	⇩800 - ⇩1700	⇩550 - ⇩750	⇩900 - ⇧650
NO^+	Affected by humidity	x	x	x	x
	Time 0 min Levels (ppb)	2500	2200 - 2700	3500 - 3800	2300 - 3500
	Time 360 min Levels (ppb)	2370	2050 - 2350	2970	2600 - 2800
	Change in concentration (ppb)	⇩130	⇩350 - ⇩650	⇩530 - ⇩830	⇩900 - ⇧500
Ethanol	Affected by humidity	x	x	x	x
NO^+	Time 0 min Levels (ppb)	3800	3300 - 4100	2700 - 2900	2300 - 2600
	Time 360 min Levels (ppb)	2700 - 2900	2700 - 3050	3350 - 3550	3200
	Change in concentration (ppb)	⇩900- ⇩1100	⇩250 - ⇩1400	⇧450 - ⇧850	⇧600 - ⇧900
H_3O^+	Affected by humidity	x	x	✓	✓
	Time 0 min Levels (ppb)	2800	3300 - 4200	4000	2300 - 2700
	Time 360 min Levels (ppb)	2000 - 2200	2650 - 3000	2400	3200
	Change in concentration (ppb)	⇩600 - ⇩800	⇩300 - ⇩550	⇩1600	⇩500 - ⇩900
Isoprene	Affected by humidity	x	x	x	x
NO^+	Time 0 min Levels (ppb)	2800	2400 - 3100	2800 - 3100	2600 - 2700
	Time 360 min Levels (ppb)	2300	2200 - 2600	2300 - 2500	2200 - 2500
	Change in concentration (ppb)	⇩500	⇩900 - ⇧200	⇩300 - ⇩800	⇩100 - ⇩500
O_2^+	Affected by humidity	x	x	x	x
	Time 0 min Levels (ppb)	2800 - 2900	2300 - 3000	3100 - 3400	2950
	Time 360 min Levels (ppb)	2350	2300 - 2700	2500	2300 - 2600
	Change in concentration (ppb)	⇩450 - ⇩550	⇩800 - ⇩1700	⇩600 - ⇩900	⇩350 - ⇩650
H_3O^+	Affected by humidity	x	x	x	x
	Time 0 min Levels (ppb)	2800	2300 - 3000	3300 - 3500	2800 - 3600
	Time 360 min Levels (ppb)	2250 - 2400	2250 -2550	2700 - 2850	2400 - 2800
	Change in concentration (ppb)	⇩400 - ⇩550	⇩750 - ⇧250	⇩450 - ⇩800	⇩0 - ⇩1200
Pentane	Affected by humidity	x	x	≡	≡
O_2^+	Time 0 min Levels (ppb)	1950	1700	1900 - 2400	1600 - 2400
	Time 360 min Levels (ppb)	1200 - 1600	1350	2650	2350
	Change in concentration (ppb)	⇩350 - ⇩750	⇩350	⇧250 - ⇧750	⇩50 - ⇧750

Table 8.2: Behaviour of compounds for relevant precursor and storage substrate and temperature where ✓ indicates an effect, x no effect and ≡ cannot say, ⇧ indicates and increase and ⇩ a decrease in concentration. Where two changes in concentration are given they are the maximum and minimum possible concentration changes given the interbag variation

The results indicate that the sample humidity and storage temperature affect the sample integrity of certain compounds. The affect of humidity is also dependent on the precursor used. Acetone and ethanol are particularly affected by the humidity and storage temperature.

8.2.3 INTER-BAG VARIATION & INTRA-BAG VARIANCE

The inter-bag variation and intra-bag variance results are shown in Table 8.3. The 37°C stored nitrogen bags for ethanol contained one bag which was injected with 1

ppm less than the others as shown in Figure 8.6. The last measurement of all compounds for the 23°C-25°C stored nitrogen substrate also had a bag whose concentration deviated from the other two bags as shown by pentane in Figure 8.7 and by the other compounds in Appendix A1.

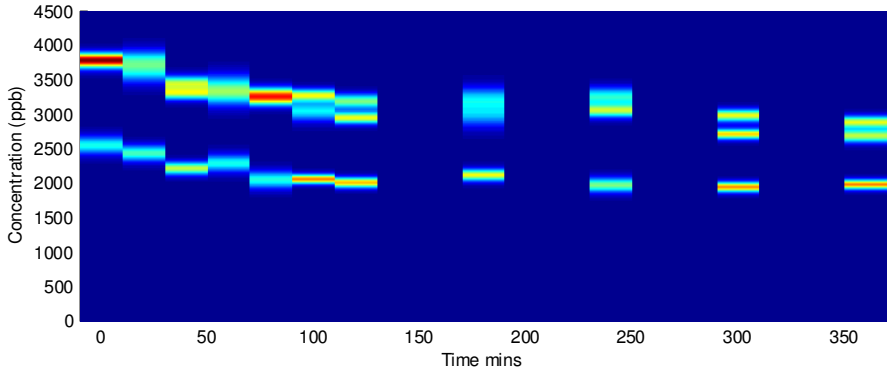


Figure 8.6: Ethanol in nitrogen filled Tedlar™ bags, stored at 37°C and monitored using the NO^+ precursor indicating that one bag was injected with 1 ppm less than the other two bags

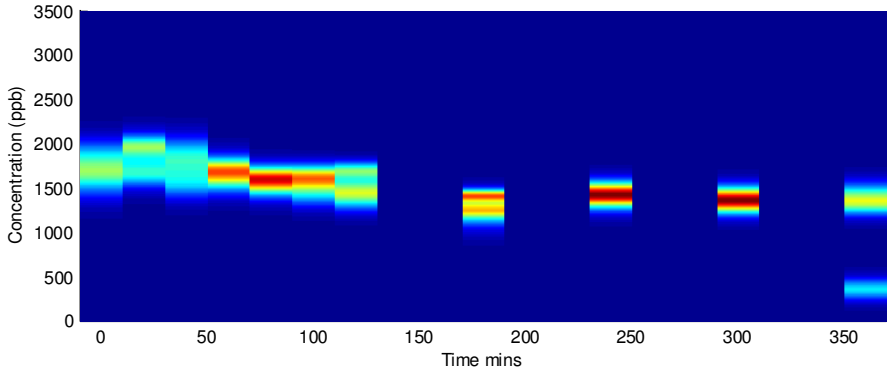


Figure 8.7: Pentane in nitrogen filled Tedlar™ bags, stored at 23°C-25°C and monitored using the O_2^+ precursor. These data indicate at a time of 350 min one Tedlar™ bag contained a different concentration to the other two Tedlar™ bags

In general, the inter-bag variation was smaller or equivalent in the nitrogen filled bags, when compared to breath. Many 23°C-25°C stored bags also contained more inter-bag variation than their 37°C stored counterparts, while the intra-bag variance was similar. For acetone and ethanol using the H_3O^+ precursor, the intra-bag variance and inter-bag variation decreased with time for the breath substrate. For pentane in the

breath substrate, only the inter-bag variation decreased with time while the intra-bag variance remained the same. For all other compounds the intra-bag variance and inter-bag variation did not fall with time. Pentane (O_2^+) had the largest intra-bag variation of any compound. It should be noted that if more bags were tested, the number of peaks in the combined distribution would likely reduce to a single peak.

Compound & Precursor	Quantity	Nitrogen Filled Bags		Breath Filled Bags	
		37℃	23℃- 25℃	37℃	23℃- 25℃
Acetone	Interbag Variation	S	L	S	L
H_3O^+	Intrabag Variance	S	S	L	L
	Interbag Variation falls in time	x	✓	✓	✓
	Intrabag Variance falls in time	x	✓	✓	✓
NO^+	Interbag Variation	S	L	L	L
	Intrabag Variance	=	=	=	=
	Interbag Variation falls in time	x	x	x	✓
	Intrabag Variance falls in time	x	x	x	x
Ethanol	Interbag Variation	L	L	S	L
NO^+	Intrabag Variance	=	=	=	=
	Interbag Variation falls in time	x	x	x	x
	Intrabag Variance falls in time	x	x	x	x
H_3O^+	Interbag Variation	L	L	S	L
	Intrabag Variance	S	S	L	L
	Interbag Variation falls in time	x	✓	✓	✓
	Intrabag Variance falls in time	x	x	✓	✓
Isoprene	Interbag Variation	S	M	L	L
NO^+	Intrabag Variance	S	L	L	L
	Interbag Variation falls in time	x	x	x	x
	Intrabag Variance falls in time	x	x	x	x
O_2^+	Interbag Variation	S	M	L	L
	Intrabag Variance	S	L	L	L
	Interbag Variation falls in time	x	x	x	x
	Intrabag Variance falls in time	x	x	x	x
H_3O^+	Interbag Variation	S	M	L	L
	Intrabag Variance	S	L	L	L
	Interbag Variation falls in time	x	x	x	x
	Intrabag Variance falls in time	x	x	x	x
Pentane	Interbag Variation	S	S	L	L
O_2^+	Intrabag Variance	L	L	L	L
	Interbag Variation falls in time	x	x	✓	✓
	Intrabag Variance falls in time	x	✓	x	x
Humidity	Interbag Variation	S	S	L	L
	Intrabag Variance	S	S	L	L
	Interbag Variation falls in time	x	x	✓	x
	Intrabag Variance falls in time	x	x	✓	x

Table 8.3: Inter-bag variation & inter-bag variance & their behaviour in time for each compound, precursor, and absolute humidity for both substrates and storage temperatures where ✓ indicates an effect, x no effect, ≡ cannot say, = equal, S small, L large and M moderate

The results indicate that for certain compounds, the inter-bag variation and intra-bag variance are affected by the sample absolute humidity and storage temperature.

8.2.4 PRECURSOR

Both the H_3O^+ and NO^+ precursors agreed for initial readings of acetone, while final readings at 360 min only agreed for the breath substrate, as shown in Table 8.2 and Appendix A1. There was good agreement of initial and final ethanol concentrations between precursors for the 23°C-25°C stored breath and nitrogen substrates, while the 37°C stored bags did not agree between precursors for either substrate, also shown in Table 8.2 and Appendix A1. The 37°C stored and 23°C-25°C stored nitrogen substrate initial and final concentrations of isoprene agreed well for all precursors used, while the 37°C stored breath substrate showed moderate agreement in initial and final concentrations between all precursors used, as shown in Table 8.2 and Appendix A1. As pentane only used one precursor, a comparison between precursors was not possible.

Overall, for most compounds the concentrations given by separate precursors agree for the 23°C-25°C stored breath and nitrogen bags, while the 37°C stored bags do not agree for either substrate.

8.2.5 STORAGE TIME

Table 8.4 summarises the changes in concentrations over the 360 min testing period, which are plotted in Appendix A1. All compounds in the nitrogen substrate except for 37°C stored acetone (NO^+) displayed losses in sample integrity with time. Acetone (NO^+) in the 37°C stored nitrogen substrate remained constant over the 360 min time

period (Appendix A1, Figure A1.3). The biggest loss of sample integrity was for acetone (H_3O^+) in the nitrogen substrate (Appendix A1, Figure A1.2). All compounds in the breath substrate displayed regular losses of sample integrity with time, except for the 37°C and 23°C-25°C stored ethanol (NO^+) (Appendix A1, Figure A1.4), 37°C stored ethanol (H_3O^+) (Appendix A1, Figure A1.4) and pentane (O_2^+) (Appendix A1, Figure A1.9).

Compound	Precursor	Nitrogen Filled Bags		Breath Filled Bags	
		37℃	23℃- 25℃	37℃	23℃- 25℃
Acetone	H_3O^+	⇩1200 - ⇩1300	⇩800 - ⇩1700	⇩550 - ⇩750	⇩900 - ⇧650
	NO^+	⇩130	⇩350 - ⇩650	⇩530 - ⇩830	⇩900 - ⇧500
Ethanol	NO^+	⇩900 - ⇩1100	⇩250 - ⇩1400	⇧450 - ⇧850	⇧600 - ⇧900
	H_3O^+	⇩600 - ⇩800	⇩300 - ⇩550	⇩1600	⇩500 - ⇩900
Isoprene	NO^+	⇩500	⇩900 - ⇧200	⇩300 - ⇩800	⇩100 - ⇩500
	O_2^+	⇩450 - ⇩550	⇩800 - ⇩1700	⇩600 - ⇩900	⇩350 - ⇩650
	H_3O^+	⇩400 - ⇩550	⇩750 - ⇧250	⇩450 - ⇩800	⇩0 - ⇩1200
Pentane	O_2^+	⇩350 - ⇩750	⇩350	⇧250 - ⇧750	⇩50 - ⇧750

Table 8.4: Change in concentration over the 360 min testing time for the relevant compound and precursor in each substrate and for each storage temperature, where ⇧ indicates and increase and ⇩ a decrease in concentration. Where two changes in concentration are given they are the maximum and minimum possible concentration changes given the interbag variation

The 23°C-25°C stored ethanol (NO^+) in the breath substrate increased steadily in time, while the 37°C stored ethanol (NO^+) in breath remained constant for 50 min and then increased to a maximum value and then fell slightly from there. However, both the bags at 23°C-25°C and 37°C, exhibited concentration increases over time for ethanol (NO^+) in the breath substrate. The 37°C stored ethanol (H_3O^+) in breath increased to a maximum of 5000 ppb over the first 50 min and then fell. The 37°C stored pentane (O_2^+) in the breath substrate had a similar behaviour to the 37°C stored and 23°C-25°C stored ethanol (NO^+), where it remained constant for the first 50 min, then increased and remained constant until time 360 min. However, due to the large

combined variation seen in pentane (O_2^+) in the breath substrate, firm conclusions are difficult to draw.

The total standard deviation, s_T, which included the between and within group variance for pentane, acetone and ethanol ranged from 217 – 350 ppb from chapter 7. Table 8.5 shows that the average change of sample integrity for pentane, isoprene, ethanol and acetone ranged from 0.2 to 3.6 times the maximum total standard deviation (350 ppb). Therefore, for most compounds, the change in sample integrity was not much greater than the largest total standard deviation. The small difference between s_T and sample integrity change makes it difficult to establish whether an actual change in sample integrity occurred.

Compound	Precursor	Nitrogen Filled Bags		Breath Filled Bags	
		37℃	23℃- 25℃	37℃	23℃- 25℃
Acetone	H_3O^+	**3.6**	**3.6**	**1.9**	**2.2**
	NO^+	**0.2**	**1.4**	**1.9**	**2.0**
Ethanol	NO^+	**2.9**	**2.4**	**1.9**	**2.1**
	H_3O^+	**2.0**	**1.2**	**2.3**	**2.0**
Isoprene	NO^+	**0.7**	**1.6**	**1.6**	**0.9**
	O_2^+	**1.4**	**3.6**	**2.1**	**1.4**
	H_3O^+	**1.4**	**1.4**	**1.8**	**1.7**
Pentane	O_2^+	**1.6**	**0.5**	**1.4**	**1.1**

Table 8.5: Number of times the average total change in compound concentration can be divided by the maximum s_T (350ppb) for each compound and precursor in both substrates and for both storage temperatures

Overall, most compounds showed a regular loss of sample integrity in time with the exception of ethanol. However, the loss of sample integrity was only marginally outside the combine repeatability and precision of the machine.

8.2.6 REPRODUCIBILITY OF RESULTS

To ensure that the results were reproducible, the behaviour was checked against the 1L bag sizes in the effect of storage size tests. Only breath substrates were used in the effect of storage size tests, as discussed in chapter 5. However, it is not necessary to verify the nitrogen substrates, as the breath substrate would have the most varied behaviour and is also more important to verify.

Both the 37°C and 23°C-25°C stored bags had similar sample integrity trends with time and similar intra-bag variation for the 1L bag sizes from the storage size tests, when compared with the sample integrity of acetone, isoprene, ethanol and pentane tests. The only difference between the two sets of tests was the inter-bag variation for the 23°C-25°C stored bags, as shown in Figure 8.8, while the 37°C stored bags showed little difference. The difference was seen for all compounds and precursors. The 1L bag sizes from the storage size tests had smaller inter-bag variation compared to the sample integrity of acetone, isoprene, ethanol and pentane tests as shown in Figure 8.8.

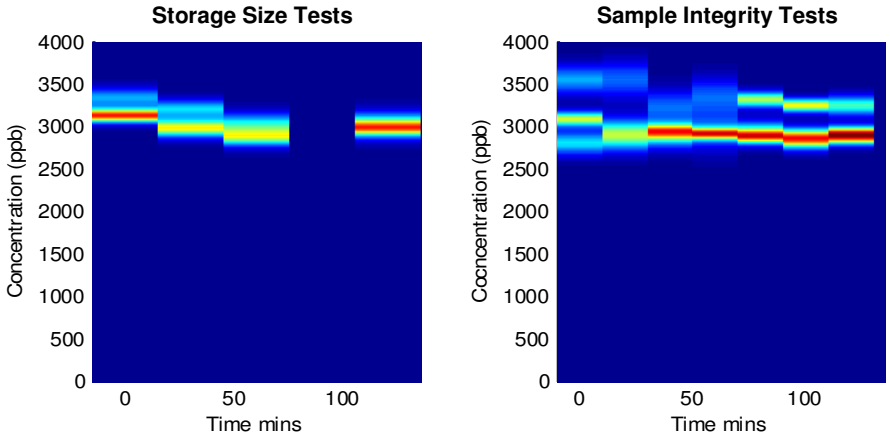


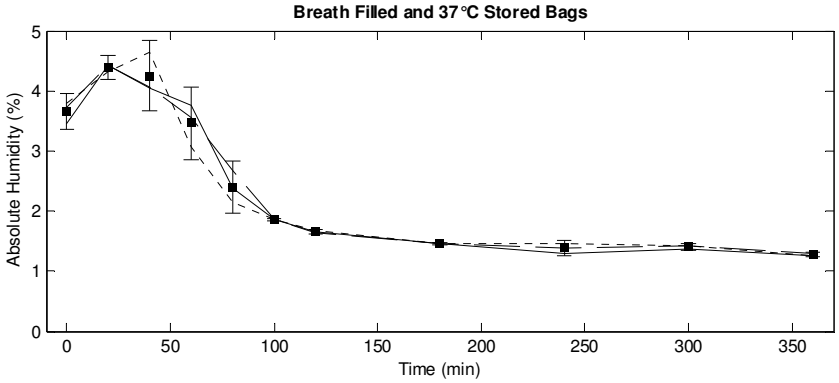
Figure 8.8: Isoprene monitored using the H_3O^+ precursor in 23°C-25°C stored breath for two seperately repeated experiments indicating the difference in inter-bag variation

8.2.7 SAMPLE ABSOLUTE HUMIDITY

The absolute humidity and behaviour of the absolute humidity was different for each substrate as seen in Figure 8.9 and Table 8.6. The nitrogen substrate tests increased their absolute humidity with time, while the breath substrates decreased their absolute humidity with time. All bags approached an absolute humidity of 1.2-1.4 %, which was most likely the atmospheric absolute humidity level. As explained in section 8.1, the initial rise in the absolute humidity of the 37°C stored breath was due to the experimental method used. The absolute humidity of the 37°C stored and breath filled bags decreased at a faster rate than the 23°C-25°C stored breath.

Quantity	Nitrogen Filled Bags		Breath Filled Bags	
	37°C	23°C- 25°C	37°C	23°C- 25°C
Time 0 min Levels (%)	0.6	0.6	3.6	3.4
Time 360 min Levels (%)	1.2	1.2	1.3	1.4
Change in humidity (%)	⇧0.6	⇧0.6	⇩2.3	⇩2

Table 8.6: Absolute humidity behaviour for substrate and storage temperature where⇧ indicates and increase and ⇩ a decrease in absolute humidity



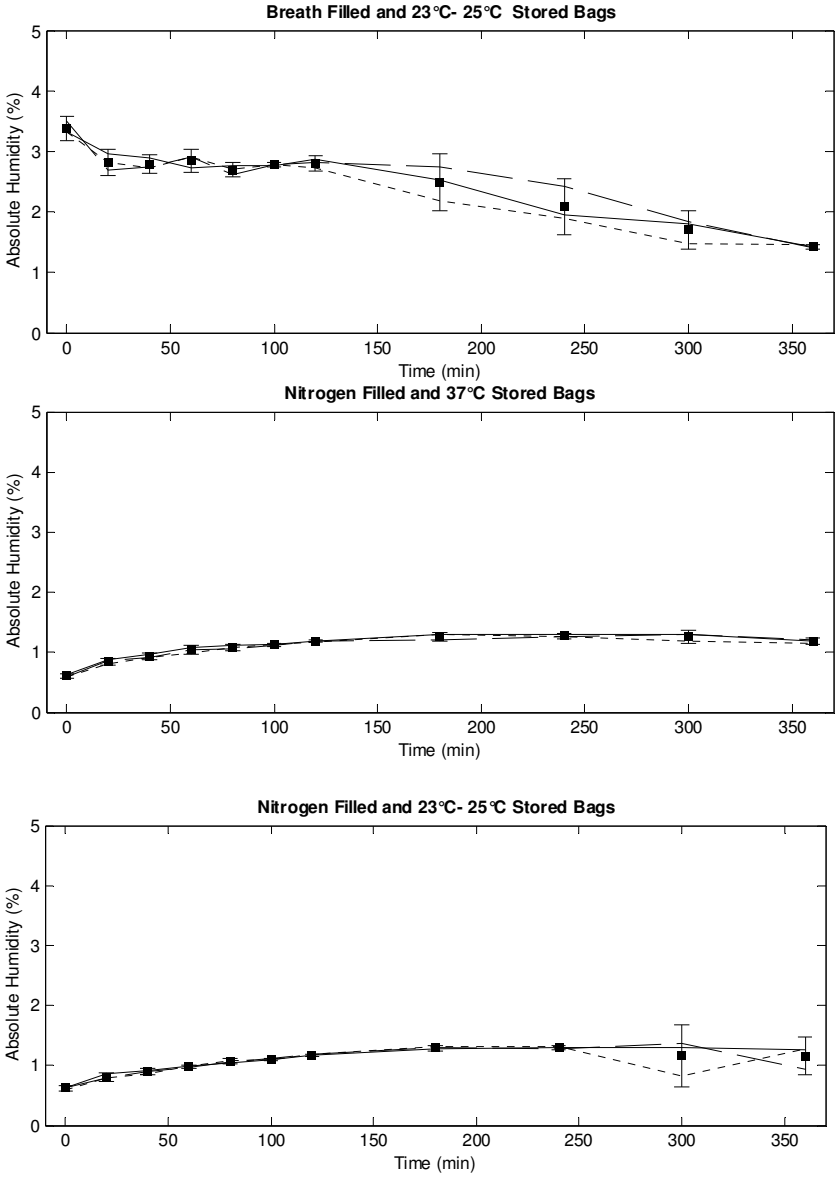


Figure 8.9: Absolute humidity change in the 1L Tedlar™ bags for each substrate and storage temperature

8.3 SAMPLE INTEGRITY OF AMMONIA

The sample integrity of ammonia was tested in separate experiments to acetone, isoprene, ethanol and pentane. The same aspects as the sample integrity of pentane, isoprene, acetone and ethanol tests were studied to establish trends in the measured

concentrations over time and to provide possible reasons for the behaviour observed. Note that the bags filled with breath and stored at 23°C-25°C did not have a measurement at time 360 min as shown in Appendix A3.

8.3.1 STORAGE TEMPERATURE AND ABSOLUTE HUMIDITY

For ammonia, the breath and nitrogen substrates displayed different concentration trends with time between the storage temperatures, as shown in Table 8.7 and 8.8. In both the 37°C and 23°C-25°C stored and breath filled bags, ammonia (O_2^+, H_3O^+) had lower starting concentrations compared to the nitrogen substrate, as shown in Table 8.7 and from the plots in Appendix A3.

Compound & Precursor	Quantity	Nitrogen Filled Bags		Breath Filled Bags	
		37℃	23℃- 25℃	37℃	23℃- 25℃
Ammonia	Interbag Variation	**L**	**S**	**L**	**L**
H_3O^+	Intrabag Variance	**=**	**=**	**=**	**=**
	Interbag Variation falls in time	**x**	**=**	**✓**	**x**
	Intrabag Variance falls in time	**=**	**=**	**✓**	**x**
	Affected by Humidity	**x**	**x**	**✓**	**✓**
	Time 0 min Levels (ppb)	**2800 - 3000**	**3050**	**800 - 1700**	**230 - 390**
	Time 360 min Levels (ppb)	**1200 - 2400**	**2400**	**2700 - 3000**	**2800 - 3800**
	Change in concentration (ppb)	**⇩400 - ⇩1800**	**⇩650**	**⇧1000 - ⇧2200**	**⇧1300 - ⇧3500**
O_2^+	Interbag Variation	**L**	**S**	**L**	**L**
	Combined Variance	**=**	**=**	**=**	**=**
	Interbag Variation falls in time	**x**	**=**	**✓**	**x**
	Intrabag Variance falls in time	**=**	**=**	**✓**	**x**
	Affected by Humidity	**x**	**x**	**✓**	**✓**
	Time 0 min Levels (ppb)	**2940 - 3300**	**3200**	**750 - 1340**	**300 - 500**
	Time 360 min Levels (ppb)	**1150 - 2300**	**2300**	**2500 - 2700**	**2800 - 3800**
	Change in concentration (ppb)	**⇩640 - ⇩2150**	**⇩900**	**⇧1160 - ⇧1950**	**⇧1300 - ⇧3500**
Humidity	Interbag Variation	**S**	**S**	**L**	**L**
	Intrabag Variance	**S**	**S**	**L**	**L**
	Interbag Variation falls in time	**=**	**=**	**✓**	**✓**
	Intrabag Variance falls in time	**=**	**=**	**✓**	**✓**
	Time 0 min Levels (%)	**0.5**	**0.4**	**4.3**	**2.9**
	Time 360 min Levels (%)	**1.4**	**1.5**	**1.6**	**1.9**
	Change in humidity (%)	**⇧0.9**	**⇧1.1**	**⇩2.7**	**⇩1**

Table 8.7: Inter-bag variation and inter-bag variance and their behaviour in time including the absolute humidity and change in concentration over the 360 min for ammonia and relevant precursor, storage substrate and temperature. Where ⇧ indicates an increase and ⇩ a decrease, ✓ indicates an effect, x no effect, = equal, S small, L large and M moderate. Where two changes in concentration are given they are the maximum and minimum possible concentration change given the interbag variation

Compound	Precursor	Different Concentration Behaviour for Breath & Nitrogen Filled bags	Different Concentration Behaviour for Storage Temperature	
			Nitrogen Filled Bags	Breath Filled Bags
Ammonia	H_3O^+	✓	✓	✓
	O_2^+	✓	✓	✓

Table 8. 8: Indication of the effect of substrate and temperature on the behaviour of ammonia for the relevant precursor, storage substrate and temperature

8.3.2 INTER-BAG VARIATION & INTRA-BAG VARIANCE

The bags filled with nitrogen and stored at 37°C had a significant increase in inter-bag variation with time as shown in Figure 8.10. Only the bags filled with nitrogen and stored at 23°C-25°C contained small inter-bag variation as shown in Figure 8.10. Generally, the intra-bag variance was comparable for ammonia between all storage temperatures and substrates.

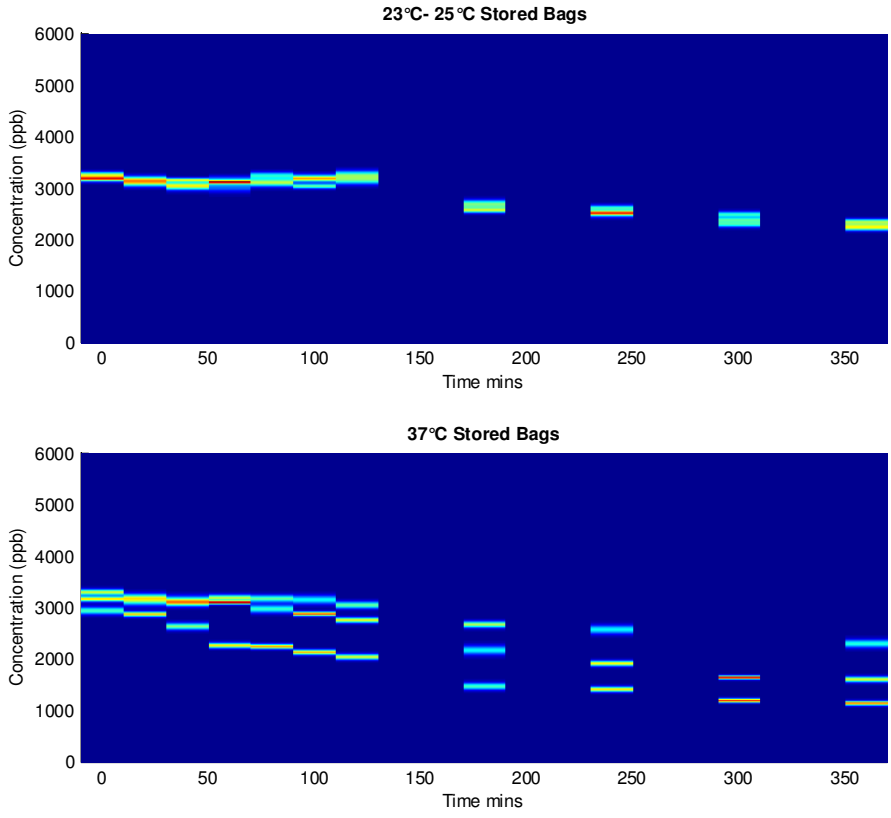


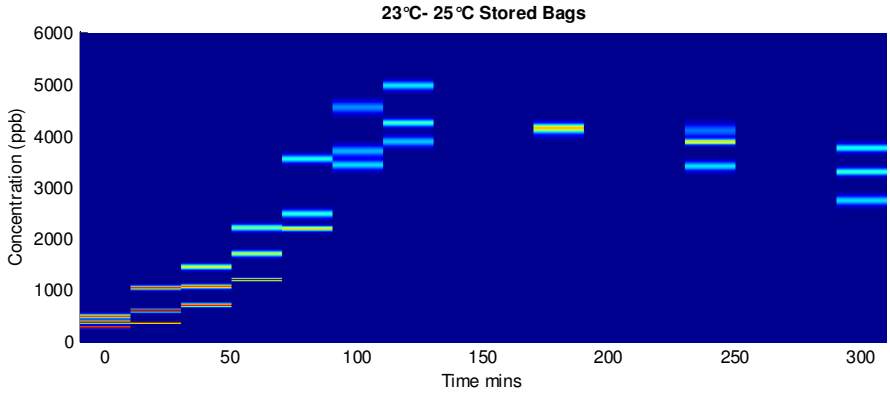
Figure 8.10: Ammonia in nitrogen filled 1L Tedlar™ bag and monitored using the O_2^+ precursor for each storage temperature, indicating the difference in inter-bag variation

8.3.3 STORAGE TIME

The bags filled with nitrogen and stored at 23°C-25°C had constant levels of ammonia, which then decayed after time 100 min as shown in Figure 8.10 and the Appendix A3. The bags filled with breath had initial increasing levels, which then decayed after a maximum ammonia concentration was reached, as shown in Figure 8.11 and the Appendix A3. This maximum concentration was reached for the 23°C-25°C stored breath and 37°C stored breath at times 120 min and 60 min respectively.

It was assumed the total standard deviation, s_T, which was found for pentane, acetone and ethanol was representative of that expected from ammonia. The average change of bag ammonia concentrations over the total testing time ranged from 0.9 to 10 times the maximum total standard deviation of 350 ppb.

The behaviour of the 23°C-25°C stored and 37°C stored breath as seen in Figure 8.11 makes it impossible to establish the concentration. More specifically, the initial concentration was supposed to be approximately 3000 ppb based on the amount injected and was measured at 1000 ppb, as indicated in Table 8.7 and Figure 8.11. This difference was not readily explained given the wide variation in behaviour observed with ammonia.



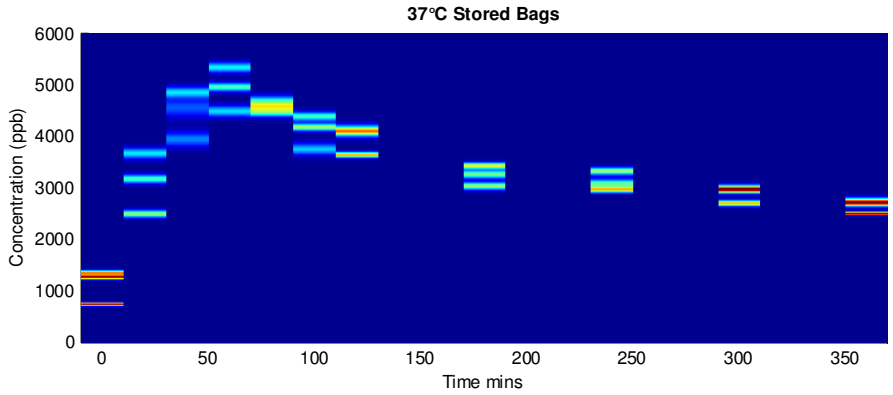


Figure 8.11: Ammonia in breath filled 1L Tedlar™ bag and monitored using the O_2^+ precursor for each storage temperature

8.3.4 REPRODUCIBILITY

Like the sample integrity of pentane, isoprene, ethanol and acetone tests, to ensure the results were reproducible the behaviour of ammonia was checked against a second experimental run done over 80 min. For both tests, the 37°C and 23°C-25°C storage temperatures had similar sample integrity trends with time and intra-bag variation. The only difference between the two experiments was the inter-bag variation for the 37°C stored nitrogen, as shown in Figure 8.12.

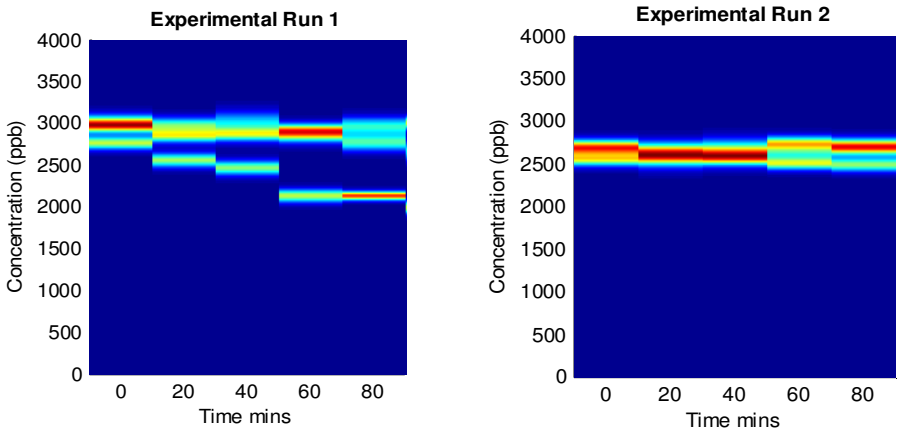


Figure 8.12: Two experimental runs for ammonia (O_2^+) showing the difference in inter-bag variation.

8.3.5 BACKGROUND PERMEATION

Background permeation tests for ammonia, as shown in Figure 8.13 indicated that the behaviour of the compounds was unlikely due to permeation of compounds from the atmosphere into the bags. Levels of compounds from time 0 to 360 min only changed by a maximum of 60 ppb for ammonia (H_3O^+) in the 23°C-25°C stored bags.

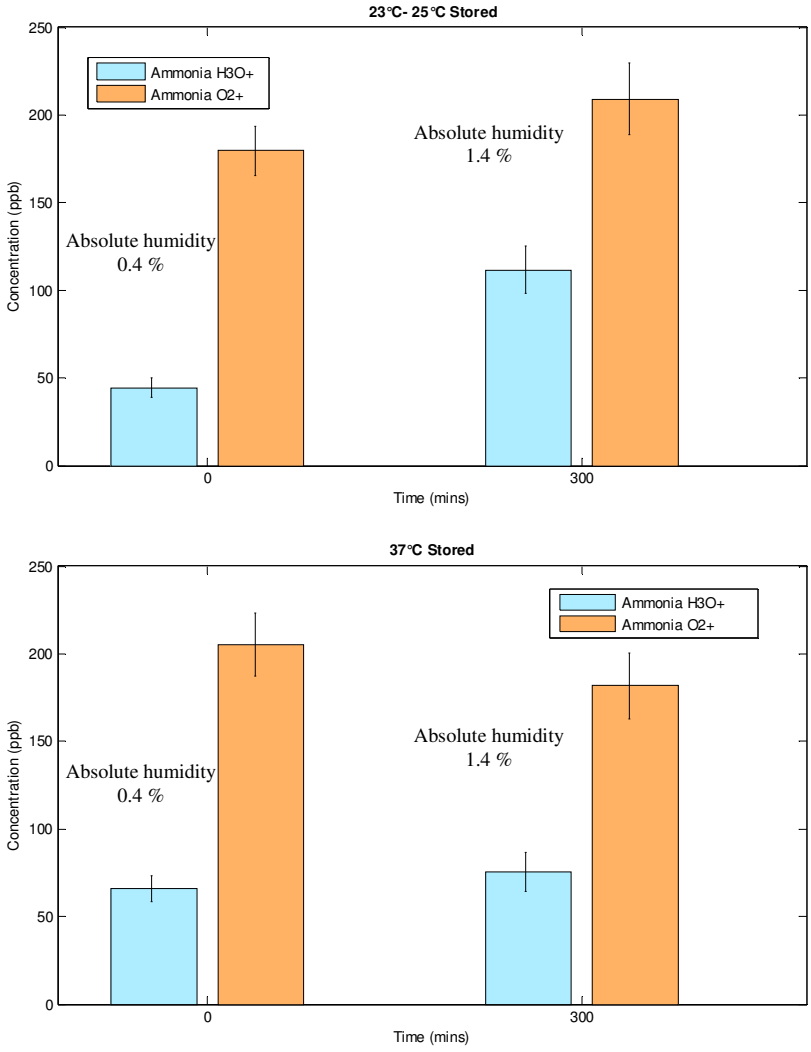


Figure 8.13: Nitrogen filled permeation test Tedlar™ bag, tested at time 0 and 360 min for the 37°C and 23°C-25°C storage temperatures

8.3.6 PRECURSOR

The 37°C stored breath bags for ammonia had higher starting concentrations than the 23°C-25°C stored breath bags for both precursors as shown in Table 8.7. The nitrogen substrate had comparable starting concentrations between precursors, also shown in Table 8.7.

8.3.7 SAMPLE ABSOLUTE HUMIDITY

The absolute humidity and behaviour of the absolute humidity was different for each substrate, as seen in Table 8.7 and (Appendix A3, Figure A3.4). The nitrogen filled bags increased their absolute humidity with time, while the breath filled bags decreased in absolute humidity with time. All bags approached an absolute humidity of 1.4-1.6 %, which was most likely the atmospheric absolute humidity level. The bags filled with breath and stored at 37°C lost their absolute humidity at a higher rate than the bags filled with breath and stored at 23°C-25°C.

8.4 SUMMARY

No obvious effects of storage size on the sample integrity of pentane, isoprene, ethanol and acetone were observed. The absolute humidity was linked to the volume to surface area ratio because it was more affected by permeation and condensation.

All compounds in the nitrogen substrate except for 37°C stored acetone (NO^+) displayed losses in analyte concentration with time. All compounds in the breath substrate displayed regular losses of analyte, except for the 37°C stored and 23°C-25°C stored ethanol (NO^+), 37°C stored ethanol (H_3O^+), pentane (O_2^+) and ammonia (H_3O^+, O_2^+). The average change over the total testing time of the concentration for

pentane, isoprene, ethanol and acetone ranged from 0.2 to 3.6 times the maximum s_T, while ammonia ranged from 0.9 – 10 times. Absolute humidity and storage temperature affected the sample integrity of acetone, ethanol and ammonia. All tests were shown to be reproducible except for the inter-bag variation in some cases. Generally the intra-bag variance was comparable between all storage temperatures and substrates while the inter-bag variation was affected by the absolute humidity. Only the initial and final concentrations between precursors for the 23°C-25°C stored breath and nitrogen substrates agreed. The breath substrate bags gave erroneous values for ammonia. Permeation of compounds into the bags from the atmosphere was not significant.

This chapter described the results from the experiments used to test the sample integrity of acetone, isoprene, ethanol and pentane, ammonia including the effect of the bag storage size on the sample integrity. The next chapter will discuss the reasons for the observed sample integrity behaviour.

9 DISCUSSION

This chapter discusses the results from the experiments in the preceding chapters. The main contributors to the sample integrity behaviour observed in were:

- physical and chemical effects of the compounds in the bags
- kinetics and other machine effects, including repeatability and precision
- experimental error from physically carrying out the experiments

These sources can be used to explain some of the behaviour seen. However, no one source is responsible for the behaviour. Instead, it is often a combination of all sources. More problematically, the contribution from each source to the overall behaviour changes over time as the variables upon which they are dependent (such as the humidity) themselves vary with time. The overall effect is thus highly non linear and difficult to predict.

9.1 PHYSICAL EFFECTS

There are many physical processes within the Tedlar™ bag and between the atmosphere and the Tedlar™ bag, as shown in Figure 9.1, that combine to affect the integrity of the samples.

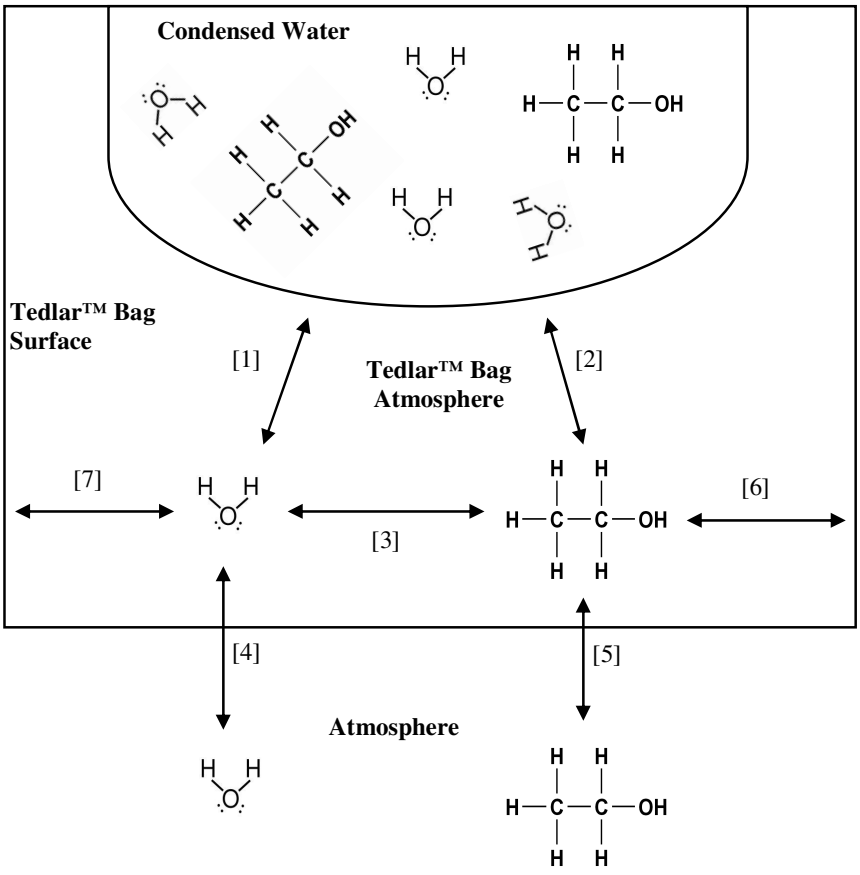


Figure 9.1: Physical processes going on within the Tedlar™ bag and between the atmosphere

[1] water condensing from vapour phase to liquid and vice versa

[2] compound moving into condensed water (solubility) and vice versa

[3] interaction of water in vapour phase with the compound in the vapour phase

[4] permeation of water in its vapour phase across the Tedlar™ bag material

[5] permeation of compound in its vapour phase across the Tedlar™ bag material

[6] interaction of compound in the vapour phase with the bag material (Tedlar™)

[7] interaction of water in the vapour phase with the bag material (Tedlar™)

The SIFT-MS technique measures the analyte concentration in the vapour phase only. Consequently, water droplets on the surface act as a sink for volatile analytes, particularly those that are hydrophilic such as ethanol and ammonia. It is clear from considering the physical processes taking place in the Tedlar™ bags, that water plays a key role in determining the measured bag concentration, especially for polar compounds that readily interact with water. The impact of water on the observed analyte concentrations in the bags is also clear from the results discussed in chapters 7 and 8, which indicate that the humidity and storage temperature of the samples noticeably affected the measured concentrations of the polar compounds acetone, ethanol and ammonia. Figure 9.2 and 9.3 demonstrate the link between the polar compound ethanol and humidity in the bags stored at both temperatures.

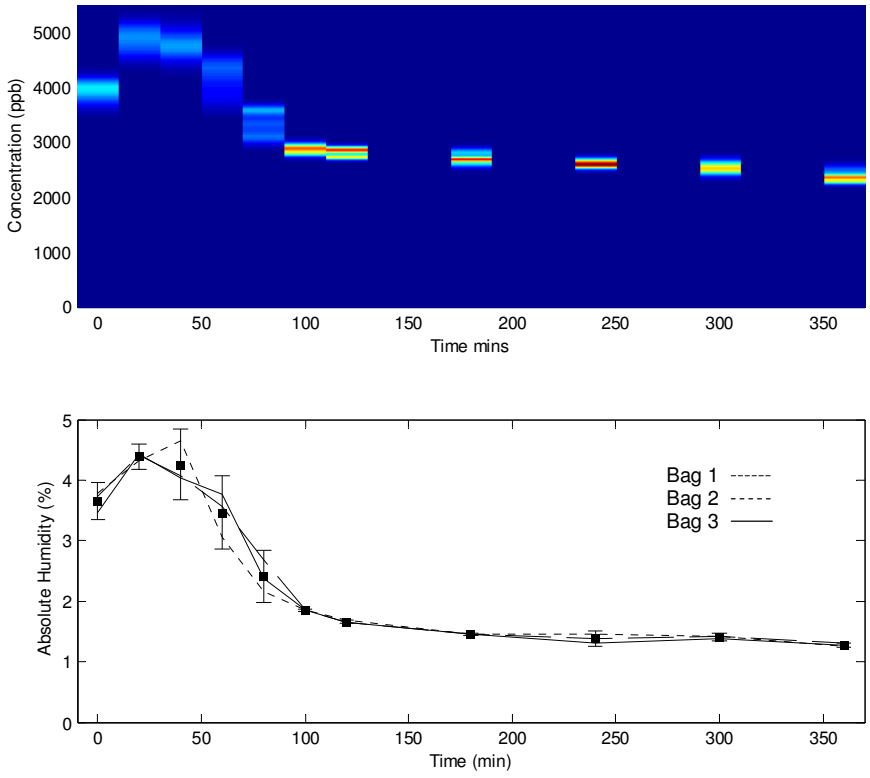


Figure 9.2: Ethanol (top) monitored using the H_3O^+ precursor in 37°C stored, breath filled 1L Tedalr™ bags showing its correlation with the absolute humidity (bottom) under the same conditions

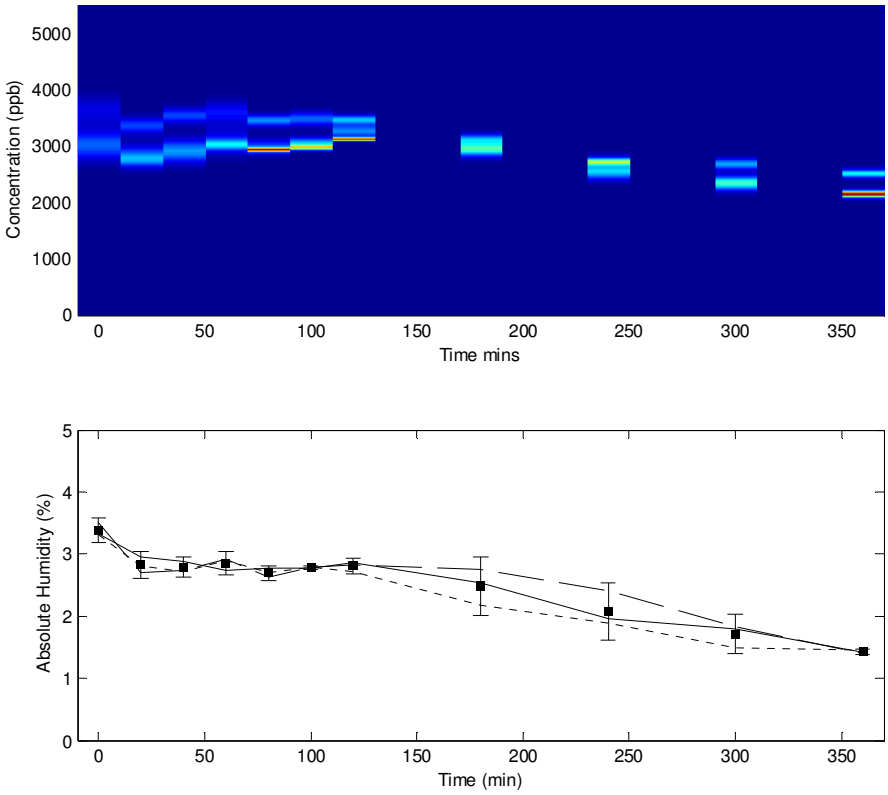


Figure 9.3: Ethanol (top) monitored using the H_3O^+ precursor in 23°C -25°C stored, breath filled 1L Tedlar™ bags showing its correlation with the absolute humidity (bottom) under the same conditions

Acetone and ethanol both had much higher concentrations in the bags filled with the breath substrate and stored at 37°C as compared to the nitrogen filled bags stored at the same temperature (Table 8.7 and Appendix A1). Ammonia had much lower starting concentrations in the breath filled bags, but over time rose to much higher concentrations. Thus, these compounds each behaves differently despite otherwise consistent, similar testing.

The effect of the storage temperature is due to its relationship to the humidity of the samples. The 37°C stored breath samples lost their humidity much more rapidly than the 23°C-25°C stored breath samples and also started at higher humidity levels. This relationship is not surprising, as heating the bag surface decreases the amount of water

condensate, which in turn increases the humidity. Studying the change in humidity of the nitrogen substrates, it is clear that the humidity of the samples is increasing. This result is most likely due to permeation of water into the bags from the atmosphere.

As water can permeate into the bag, it should also be able to permeate out of the bag. Therefore, the likely loss of humidity from the breath filled bags was due to permeation, especially as their temperatures were kept constant and condensation would therefore be unaffected. The driving force for the humidity is therefore the difference between the humidity of the bag and the atmosphere. This effect was seen with the breath substrate samples falling and nitrogen substrate samples rising to the same humidity of 1.2 – 1.4% over the 360 min testing time as shown in Figure 8.9.

If water was permeating through the bags, then it was possible for other compounds to also permeate through the bags. This could therefore be a possible explanation for the observed behaviour. Background permeation tests for ammonia, isoprene, ethanol, acetone and pentane, as shown in Figures 8.5 and 8.13, indicated that the behaviour of the compounds was unlikely to be due to permeation of compounds from the atmosphere into the bags. Levels of compounds from time 0 to 360 min only changed by a maximum of 180 ppb, which was not significant compared to the measured decay, and were within the total standard deviation, s_T range of 217 – 350 ppb. The validity of the 180 ppb change is also questionable considering that the initial levels of the compounds were between 50 – 200 ppb when none should have been present in the pure nitrogen sample.

The comparison of storage size indicated that the volume to surface area ratio had minimal effect on the sample integrity of acetone, ethanol, isoprene and pentane. As changing the volume to surface area ratio would influence the permeation, the little difference observed between bag storage sizes on the sample integrity of compounds indicates that permeation out of the bag is perhaps not significant for pentane, isoprene, ethanol and acetone. However, if ammonia had been tested it would have most likely shown an effect with changing volume to surface area ratio based on its size. Specifically, the ammonia molecule has a molecular mass of 17 amu, which is smaller than water's molecular mass of 18 amu. As permeation is largely reliant on the molecule size, any molecule of a similar size to water would also permeate through or interact with the bag.

The model behaviour of the bags filled with nitrogen for all compounds was expected due to the small amount of water present in the samples. The only exceptions were acetone when monitoring with the H_3O^+ precursor which had a significant, yet well defined, loss of concentration and the 37°C stored ammonia which had large inter-bag variation. As discussed in chapter 2, the H_3O^+ precursor is more affected by the presence of water due to the formed hydrated hydronium ions $H_3O^+.(H_2O)_{1,2,3}$ and as the observed behaviour for ethanol was not seen with the NO^+ precursor, the reason for the behaviour may be due to this effect.

The inter-bag variation and intra-bag variance was less in the nitrogen filled bags compared to breath filled bags due to the differences in complexity of the substrates. The breath substrate contained many more constituents, of which the main cause of the increased inter-bag variation and intra-bag variance was the water. The greater

inter-bag variation of the 23°C-25°C stored bags could also be linked to the humidity, as only the 37°C stored bags could ensure that all the water was present in vapour form.

The decrease of the intra-bag variance and inter-bag variation over time for acetone and ethanol and ammonia can also be explained by the humidity. As the humidity of the bags fell, there was less water available for the ethanol, acetone and ammonia to interact with and result in variation. For all the non-polar compounds, the intra-bag variance and inter-bag variation did not decrease with time, with the exception of pentane, because being non-polar meant they were unaffected by the humidity.

The reasons for the increasing concentration of ethanol, as given by the NO^+ precursor in both the 23°C-25°C stored and 37°C stored breath samples, as shown in Figure 9.4 is unclear. The behaviour could be due a physical process. Initially, there would be a set amount of condensed water and ethanol dissolved into it. As the humidity falls, water would move into the vapour phase to maintain equilibrium. As the amount of liquid water falls the amount of ethanol dissolved into the liquid water reduces, as it moves into its vapour phase. Therefore, the concentration of ethanol increases as it leaves the condensed water. This behaviour was not seen for the H_3O^+ precursor, shown in Figure 9.2 and 9.3, but as discussed, the H_3O^+ precursor is more affected by the humidity, and therefore the behaviour may be a result of its humidity dependence, as opposed to any physical reason.

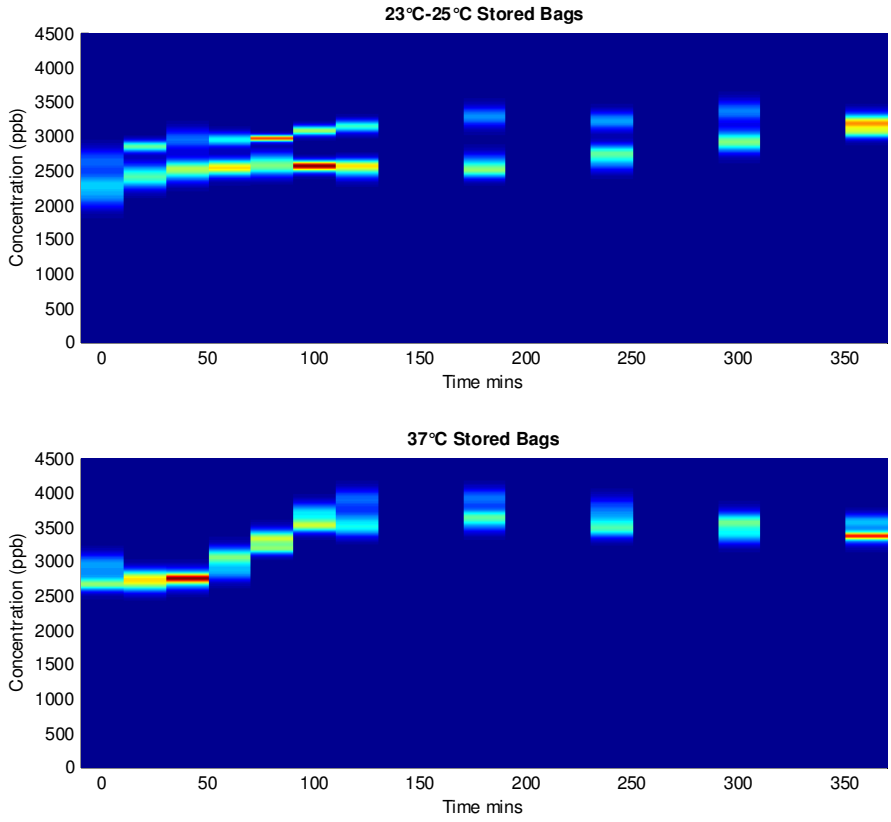


Figure 9.4: Ethanol monitored using the NO^+ precursor in breath filled 1L Tedlar™ bags for both storage temperatures

9.2 KINETICS & MACHINE EFFECTS

For the 23°C-25°C stored bags, all compounds agreed well between both substrates and all precursors, with only ethanol having lower starting concentrations in the 23°C-25°C stored breath compared to the 23°C-25°C stored nitrogen. The reason why the 23°C-25°C stored bags agreed for all compounds between both substrates and between all precursors, was due to the calibration used to determine the reaction rate coefficients.

As discussed in chapter 6, the calibration for the kinetics was carried out at room temperature (23°C-25°C) for both breath and nitrogen substrates. Therefore the

concentrations at room temperature (23°C-25°C) agreed for both substrates and all precursors. When the same kinetics were used to analyse the 37°C stored bags, it resulted in disparities between the breath and nitrogen substrates and precursors used. However, the kinetics caused greater disparities for the polar compounds ammonia, acetone and ethanol, while the disparities were less significant for non polar compounds, such as isoprene. Because the kinetics showed larger disparities between the nitrogen and breath substrates and precursors used for the polar compounds, it suggests that the humidity is a possible reason for the failure of the kinetics. It is therefore useful to understand how the kinetics are affected by the sample humidity. The relationship between the kinetics and humidity can be illustrated by studying the analysis of ethanol in breath stored at 37°C and monitored using the H_3O^+ precursor.

The masses monitored as part of the kinetics for ethanol in the breath substrate and 37°C stored condition using the H_3O^+ precursor can be plotted, as shown in Figure 9.5 and 9.6.

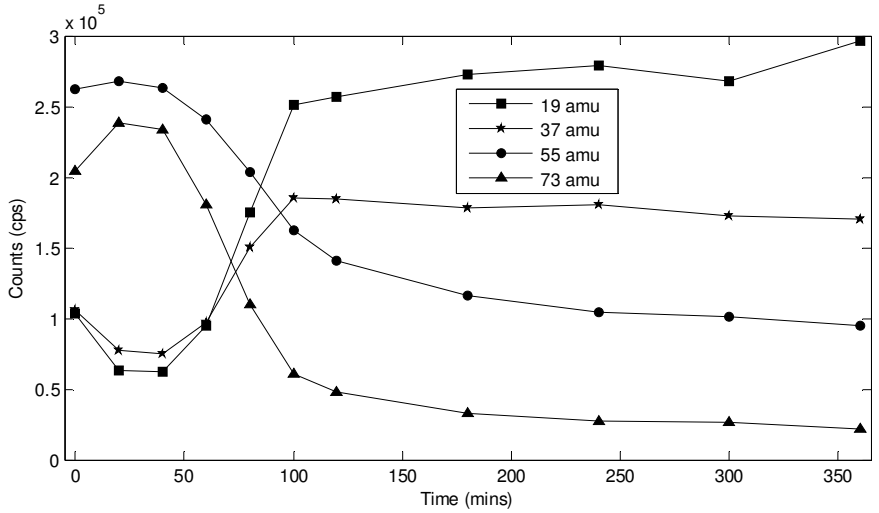


Figure 9.5: Precursor masses monitored in the breath filled Tedlar™ bags and 37°C storage condition using the H_3O^+ precursor

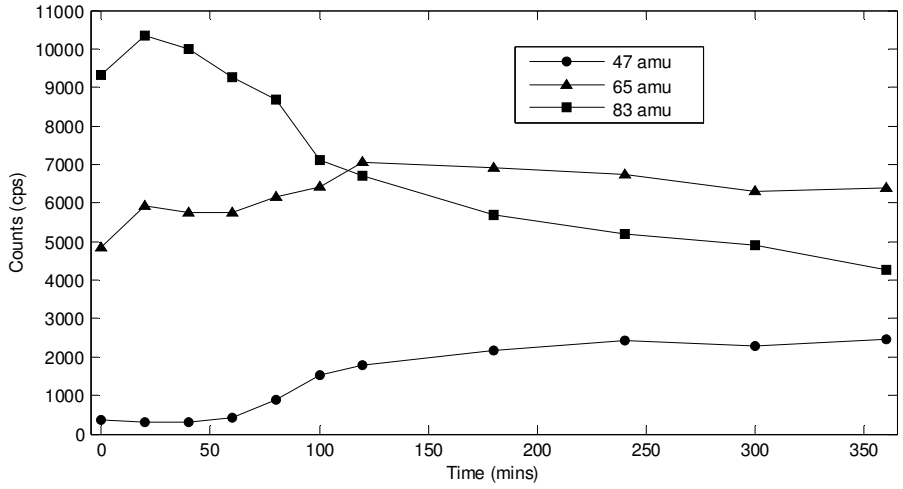


Figure 9.6: Product masses monitored in the breath filled Tedlar™ bags and 37°C storage condition using the H_3O^+ precursor

Initially, when the sample is more humid, the ethanol within the Tedlar™ bag has much more water in the vapour phase available to cluster with and hence the product mass 83, which forms from reactions of $C_2H_5OH_2.H_2O$ and H_2O (as shown in Table 9.1) is large. Within the flow tube, the large amount of water in the sample also results in the H_3O^+ precursor clustering with the water to give the 73, 55 and 37 masses, which form from $H_3O^+.H_2O$, $H_3O^+.(H_2O)_2$ and $H_3O^+.H_2O$. Conversely, as the majority of the H_3O^+ precursor ions and protonated ethanol has clustered with the water, there is not much of the un-clustered proton-bound ethanol dimer ($C_2H_5OH.C_2H_5OH_2^+$) and un-clustered H_3O^+ precursor available to form the products represented as masses 47, 65, 19 and 37 respectively. As the humidity of the sample decreases with time, the masses 83, 73, 55 also decrease, while the masses 19, 37, 47 and 65 increase. Thus it can be seen that for the H_3O^+ precursor, there is a strong link between the kinetics and the sample humidity.

Product Mass (amu)	Type	Reactants	Products
19	Precursor	none	H_3O^+
37	Precursor	H_3O^+ , H_2O	$H_3O^+.H_2O$
55	Precursor	$H_3O^+.H_2O$, H_2O	$H_3O^+.(H_2O)_2$
73	Precursor	$H_3O^+.(H_2O)_2$, H_2O	$H_3O^+.(H_2O)_3$
47	Product	H_3O^+ , C_2H_5OH	$C_2H_5OH_2^+$
65	Product	$C_2H_5OH_2^+$, H_2O	$C_2H_5OH_2^+.H_2O$
83	Product	$C_2H_5OH_2^+.H_2O$, H_2O	$C_2H_5OH_2^+.(H_2O)_2$

Table 9.1: Reactants and products for the precursor and product masses monitored for ethanol in the breath filled Tedlar™ bags and 37°C storage condition using the H_3O^+ precursor

The behaviour seen by the product and precursor masses monitored for ethanol can also be seen for the other polar compounds, ammonia and acetone. As discussed, the calibration method could not be performed for ammonia in bags filled with breath due to concentration fluctuations. Ammonia was therefore only calibrated for bags filled with nitrogen, which explains the unusual behaviour observed with ammonia in the bags filled with breath, as seen in Figure 9.7. The effect of the calibration is proven when looking at the concentration of ammonia in time. As time increases and the humidity falls, the concentration measured approaches that of the nitrogen filled bags for both storage temperatures. In Figure 9.7, the bags containing breath reach a final concentration of 2500 – 2800 ppb, while those filled with nitrogen reach 2400 ppb. The kinetics, which were calibrated for dry nitrogen samples, therefore become more appropriate and accurate as the breath sample humidity approaches that of the nitrogen samples.

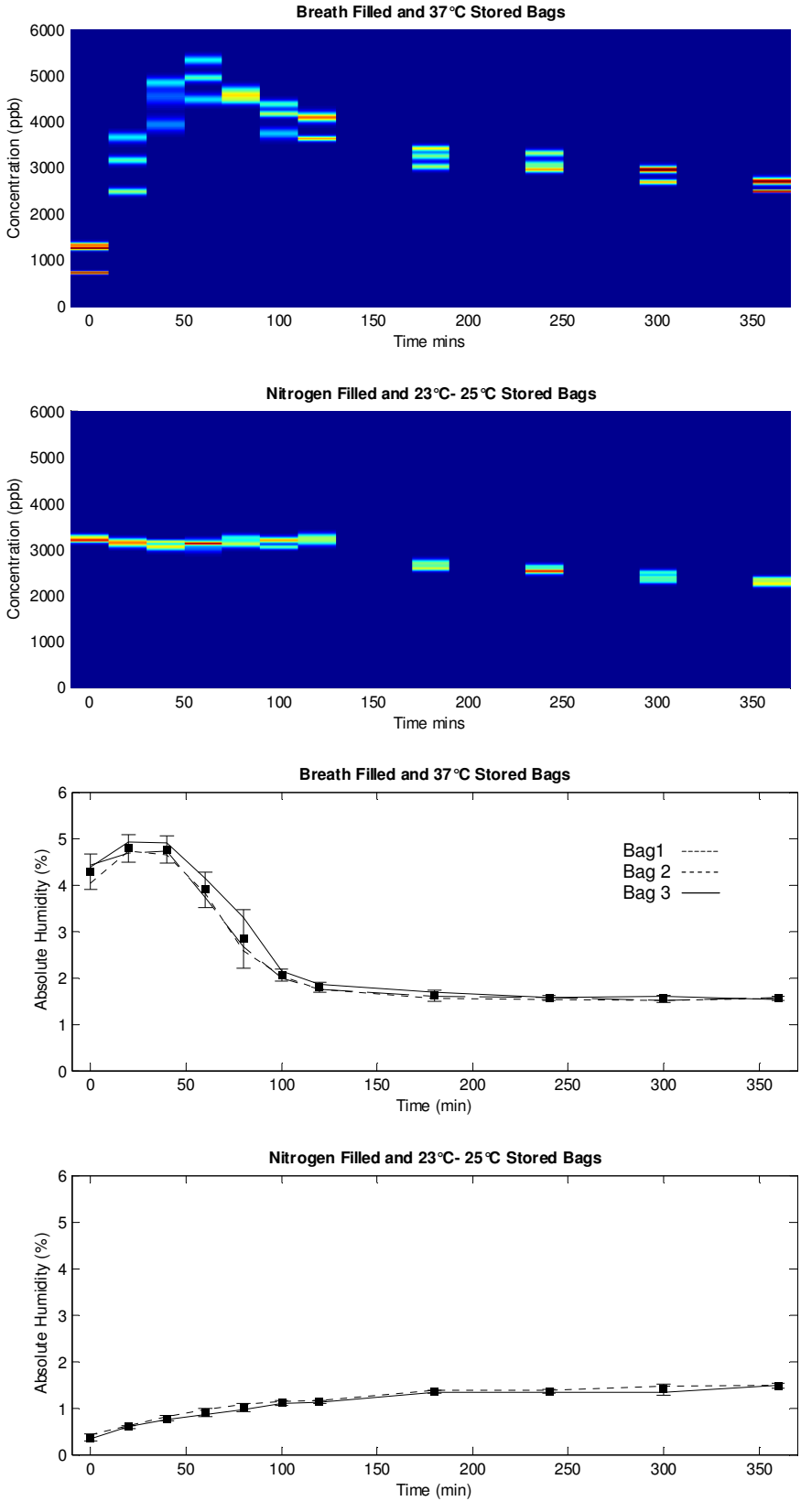


Figure 9.7: Ammonia monitored using the O2+ precursor in 1L Tedlar™ bags for the given substrate and storage temperature including the absolute humidity

More specifically, the kinetic scheme does not include liquid phase chemistry. The ion-chemistry of the polar compound's reactions with water would need to be modified for liquid water. Therefore the kinetics are not compensating correctly for the change in humidity. The complications due to humidity in the sample do not allow adequate monitoring of breath samples in Tedlar™ bags at breath humidity levels of 5-6%. The complications in the kinetics due to humidity are less significant and less pronounced for non polar compounds such as isoprene. For this reason the kinetics are more adequate in the tests done for isoprene.

Using the software developed it is possible to re-calibrate the reaction rate coefficients and re-analyse the data. The re-calibration was done by using the 37°C stored and nitrogen filled bags as the control case and minimising the differences between it and the bags containing breath. When this was done, all four reaction rate coefficients calculated to be roughly equivalent at 2×10^{-9}. When these new reaction rate coefficients are used, the large concentration values before 100 min in Figure 9.2 are reduced to more realistic levels, as shown in Figure 9.8.

The new method for calculation of the concentration of polar analytes using H_3O^+ for humid samples suggested by Spanel and Smith (2000) was also implemented. This task was performed using the existing reaction rate coefficients and the ones given by Wilson et al (2001). Using the reaction rate coefficients given by Wilson et al (2001) and the original rate coefficients in the new method for calculation given by Spanel and Smith (2000) gives results very close to the reaction rate coefficients recalibrated to the nitrogen substrate. This result is illustrated in Figure 9.9.

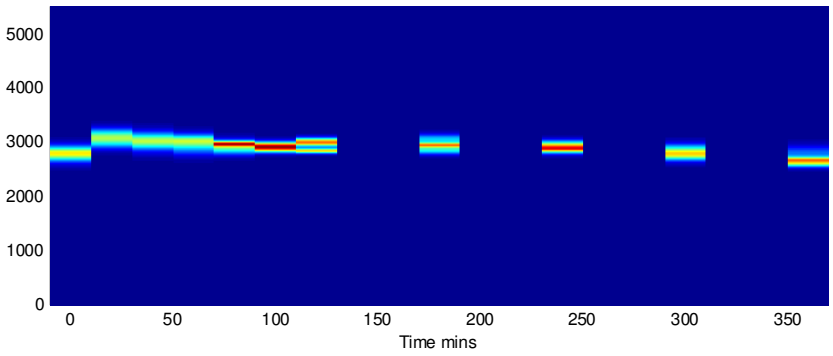


Figure 9.8: Ethanol monitored using the H_3O^+ precursor in 1L Tedlar™ bags, 37°C storage condition utilising the reaction rate coefficients recalibrated to the nitrogen substrate for the same storage temperature

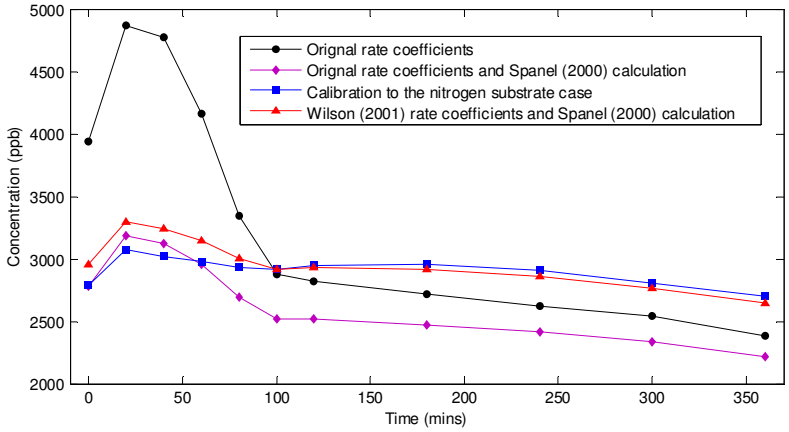


Figure 9.9: Ethanol monitored using the H_3O^+ precursor in 1L Tedlar™ bags, 37°C storage condition for different reaction rate coefficients and calculation methods

However, thought must be given to altering the reaction rate coefficients, as they become purely empirical and are no longer representative of the chemistry, and are not universally applicable. Recalibrations in this manner are not really making use of the underlying chemistry of SIFT-MS technology - chemical kinetics. Therefore the question arises of why use it at all.

The re-calibration was not successful for ethanol in the bags filled with breath, stored at 37°C stored and monitored using the NO^+ precursor as seen in Figure 9.4. The unsuccessful calibration indicates that perhaps there are more reactions taking place

that are not accounted for. This possibility applies to all compounds, as the kinetics not only involve the reaction rate coefficients, but also which reactions to monitor and their products. The branching ratios are an unlikly reason for the unsuccessful recalibration as they are generally well established. The best start is to check the current kinetics by calibrating the for breath in bags at absolute humidity levels of 6%.

Pentane had the largest within group variance, s_W, of any compound at 349 ppb, but the best between group variance, s_B. Due to the large s_W, as shown in Figure 9.10 and the other plots in Appendix A4, it is difficult to determine any trends or draw very conclusive results. Pentane, being the least polar compound, was expected to be the best behaved. As discussed in chapter 6, ideally the monitoring of pentane would involve masses 42, 43, 57, 72, 97 and 71, while only the 71 and 72 masses were monitored and their rate coefficients were multiplied by factors to account for missing masses. If all the necessary masses were monitored, the s_W would be reduced and a more defined behaviour would occur.

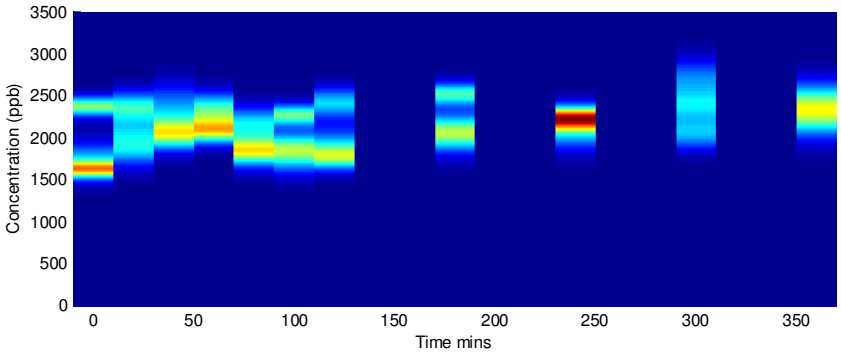


Figure 9.10: Pentane monitored using the O_2^+ precursor in 1L Tedlar™ bags filled with breath and stored at 23°C-25°C

9.3 EXPERIMENTAL ERROR

The lack of reproducibility in the inter-bag variation for the 23°C-25°C stored breath substrates between the 1L bag sizes from the storage size tests compared to the sample integrity of acetone, isoprene, ethanol and pentane tests is due to the presence of water vapour. The 23°C-25°C stored bags would not ensure that all the water was present in vapour form and would therefore be more likely to have varying amounts of humidity from bag to bag. However, because the difference in inter-bag variation was seen for all compounds and precursors, and not just polar compounds or the H_3O^+ precursor, the inter-bag variation may also be a result of the experimental error.

The large inter-bag variation seen for 37°C stored ammonia in the nitrogen substrate is possibly due experimental error, because the retests done over the first 80 mins did not indicate such large inter-bag variation.

9.4 SUMMARY

The behaviour observed was explained by looking at the physical and chemical effects of the compounds in the bags, the kinetics, and other machine effects including repeatability, precision, and the experimental error.

Humidity and storage temperature affected the sample integrity of the polar compounds acetone, ethanol and ammonia. The storage temperature effect was due to its relationship with humidity. Permeation through the bags was the cause of the humidity behaviour but was unlikely to be the cause of the compound behaviour. The humidity also caused the small inter-bag variation in the nitrogen filled bags compared to breath fileld bags and the larger inter-bag variation of the 23°C-25°C

stored samples than their 37°C stored counterparts. The lack of reproducibility was most likely due to experimental error.

Only the initial and final concentrations between precursors for the 23°C-25°C stored breath and nitrogen substrates agreed. The agreement was because the kinetics calibration was carried out at room temperature (23°C-25°C) for both the breath and nitrogen substrates. As ammonia was only calibrated in the nitrogen substrate, the breath substrate samples gave erroneous values.

The kinetic scheme does not include liquid phase chemistry, and therefore the ion-chemistry of the polar compounds reactions with water need to be modified for liquid water. The complications due to humidity in the sample do not allow adequate monitoring of breath samples in bags at breath humidity levels of 5-6%. However, the complications in the kinetics due to humidity are less significant and less pronounced for non polar compounds such as isoprene.

This chapter discussed the reasons for the observed sample integrity behaviour from the experiments undertaken. The next chapter will summarise the work undertaken in this thesis and answer the question as to the suitability of Tedlar™ bags for remote collection and storage of breath samples for later analysis using SIFT-MS.

10 CONCLUSION

This thesis examined all the relevant aspects of using a collection media to store collected breath and quantifying its variability and suitability as a sampling method for breath and later analysis using SIFT-MS.

10.1 SAMPLE STORAGE MEDIA

The use of SIFT-MS instrumentation at the Christchurch Hospital for research into disease diagnosis using breath has created a need for remote breath sampling. An important aspect of any remote sampling device is the storage medium used to collect breath samples. Tennax, SPME and Tedlar™ bags were considered storage methods. Tedlar™ bags were chosen due to their simplicity in the sampling and quantification processes.

10.2 EXPERIMENTAL

To establish the sample integrity of the samples stored in Tedlar™ bags, the effect of storage time, storage temperature, characteristics of stored compounds, absolute humidity, inter-bag variation and stored sample size on sample integrity were tested. The sample integrity was studied by studying changes in stored compound concentrations and sample humidity with time. As no study on the repeatability of

SIFT-MS has been reported, a reasonable estimate of the repeatability and precision of LDI2 was needed to achieve the end goals of the research.

Four main experiments were undertaken to establish the integrity of samples stored in Tedlar™ bags:

- Effect of storage size (0.5, 1, 3L), on the sample integrity of pentane, isoprene, ethanol, acetone in a breath substrate stored at room temperature (23°C- 25°C) and 37°C over 48 hours.
- Sample integrity of ammonia in breath and nitrogen substrates stored at room temperature (23°C- 25°C) and 37°C using 1L Tedlar™ bags over 6 hours.
- Sample integrity of pentane, isoprene, ethanol, acetone in breath and nitrogen substrates stored at room temperature (23°C- 25°C) and 37°C using 1L Tedlar™ bags over 6 hours.
- Repeatability and precision of SIFT-MS using ethanol, pentane and acetone over 250 min.

The concentration of the chosen compounds was 3ppm and each testing situation had triplicate bags.

10.3 ANALYSIS

A generalised Cauchy distribution was used to give a combined distribution from multiple bags for the sample humidity and compound concentration. The distribution included a combined mean and variance. A total "standard deviation" about the combined mean, s_T, was used as a measure of the combined effect of the precision

and repeatability. Software was written using Visual Basic and Matlab to optimise all aspects of the concentration and absolute humidity calculation, and the final results post processing.

10.4 MACHINE REPEATABLILITY AND PRECISION RESULTS

The s_T values ranged between 217 – 349 ppb for ethanol, acetone and pentane. Pentane (O_2^+) had the lowest precision, possibly because only the 71 and 72 masses were monitored in the pentane kinetics. The factors affecting the repeatability and precision of the concentration measurements, $[A]_{ppb}$, were machine dependant (T_g, Φ_{Total}, p_g) and compound dependant ([B]). The p_g and [B] accounted for 95% of the precision in $[A]_{ppb}$. The magnitude of the contribution of all factors to the precision $[A]_{ppb}$ differed in time, with different precursor and compound. All factors, except for the [B] pentane O_2^+, could be the cause of the poor repeatability in $[A]_{ppb}$, in addition to any experimental error.

10.5 SAMPLE INTEGRITY RESULTS

All compounds in the nitrogen substrate except for 37°C stored acetone (NO^+) displayed losses in sample integrity with time. All compounds in the breath substrate displayed regular losses of sample integrity, except for the 37°C and 23°C- 25°C stored ethanol (NO^+) and 37°C stored ethanol (H_3O^+), pentane (O_2^+) and ammonia (H_3O^+, O_2^+). The change of sample integrity over the total testing time for pentane, isoprene, ethanol and acetone ranged from 0.2 to 3.6 times the maximum s_T, while ammonia ranged from 0.9 – 10 times.

No obvious effects of bag storage size on the sample integrity of pentane, isoprene, ethanol and acetone were observed. However, the absolute humidity is affected by the storage size with its behaviour linked to the volume to surface area ratio. Permeation through the bags is the cause of the absolute humidity behaviour, but is unlikely to be the cause of the compound behaviour.

All tests were reproducible except for the inter-bag variation for the 23°C- 25°C stored cases and ammonia (H_3O^+, O_2^+) in the 37°C stored nitrogen substrate, which is most likely due to experimental error.

Absolute humidity and storage temperature affects the sample integrity of the polar compounds acetone, ethanol and ammonia. The storage temperature effect is due to its link with absolute humidity. The absolute humidity also affects the inter-bag variation with greater absolute humidity levels having greater inter-bag variation for polar compounds.

The initial and final concentrations between precursors for the 23°C- 25°C stored breath and nitrogen substrates agreed because the kinetics calibration was carried out at room temperature (23°C- 25°C) for both substrates. As ammonia was only calibrated in the nitrogen substrate, the breath substrate samples gave erroneous values. The kinetic scheme does not include liquid phase chemistry, and therefore the ion-chemistry of the polar compounds reactions with water need to be modified for liquid water. The complications due to humidity in the sample do not allow adequate monitoring of breath samples in Tedlar™ bags at breath humidity levels of 5-6%.

However, the complications in the kinetics due to humidity are less significant and less pronounced for non polar compounds such as isoprene.

10.6 CONCLUSION

The question of using Tedlar™ bags to store breath samples for analysis using SIFT-MS has been answered. The problem of storing breath in Tedlar™ bags for analysis using SIFT-MS is not the loss of sample integrity, but the kinetics, precision and repeatability of the SIFT-MS instrument. Generally, the loss of sample integrity was only marginally outside the repeatability and precision of the machine. The current kinetics are not adequate to accurately monitor acetone, isoprene, pentane, ammonia and ethanol in breath and stored in Tedlar™ bags at breath absolute humidity levels greater than 3%.

This chapter summarised the work undertaken in this thesis and answered the question as to the suitability of Tedlar™ bags for remote collection and storage of breath samples for later analysis using SIFT-MS. The next chapter will give recommendations for future work.

11 FUTURE WORK

Although this thesis covered a number of aspects that affected the integrity of samples stored in Tedlar™ bags, there were some aspects not studied due to the scope of the research. However, before any conclusive study into Tedlar™ bag sample integrity, the repeatability, precision and kinetics of SIFT-MS instrumentation needs to be better established.

11.1 SIFT- MS KINETICS

It is not possible to test the integrity of breath samples stored in Tedlar™ bags using SIFT-MS, if SIFT-MS is not capable of accurately measuring the studied compound concentrations. The research undertaken in this thesis indicated that the current kinetics are not adequate to monitor the studied compounds in a breath substrate, stored in Tedlar™ bags and at changing absolute humidity levels varying between 0 – 4.5%. The kinetics needs to include liquid phase chemistry, in order to overcome the complications due to humidity.

11.2 REPEATABILITY & PRECISION

Like any sensor, it is important that the repeatability and precision of any SIFT-MS instrument is known. Knowing the repeatability and precision becomes mandatory if

the measurements are used for disease diagnosis or research for medical applications. Therefore, extensive studies on the repeatability of SIFT-MS for different compounds at a range of concentrations in breath, at varying absolute humidity levels and over an adequate time period need to be conducted. To ensure a proper bias measurement can be made, the method should use samples whose concentrations are truly known. Changes and improvements to SIFT-MS technology are inevitable, but the repeatability and precision will always need to be known for any future generation machines. Therefore, if a robust process for establishing the repeatability and precision is developed, it can be implemented for any future machine updates.

11.3 SAMPLE INTEGRITY TESTING

If Tedlar™ bags are to ever be used for disease diagnosis, experiments involving up to hundreds of bags should be conducted. Using sample integrity information from a large number of bags will ensure all possible compound behaviour is accounted for. A more robust experimental method for creating the bag samples and heating them during testing would be beneficial. These improvements will ensure experimental error has minimal effect on the combined compound behaviour.

Instead of sequentially sampling from the same Tedlar™ bag, tests should be done where samples are taken once from different bags at each sampling period. Sampling from different bags at each sampling period is more applicable to what would occur in clinical reality as samples would be filled and left until tested.

Only one concentration was tested in the research presented. However, it would be beneficial to determine the effect of the compound concentration on the integrity of samples stored in Tedlar™ bags. More detailed study into the physical and chemical

properties of Tedlar™ including their adsorption and diffusion characteristics would also be beneficial.

Finally, thought should be given to the expected concentration levels in disease. If these concentrations are substantially greater than concentrations in healthy individuals, then the small loss of sample integrity from Tedlar™ bags observed in this thesis over the time tested may be irrelevant. However, in cases with significant overlap, sample integrity will be paramount.

11.4 SUMMARY

It is important at this point in its development to establish the repeatability and precision of SIFT-MS, and ensure the kinetics are capable of accurately monitoring all desired compounds in breath and stored in Tedlar™ bags, at expected ranges of absolute humidity. Once the repeatability, precision and kinetics are better established, the integrity of samples stored in Tedlar™ bags can be more properly tested and more accurately ascertained.

12 APPENDIX

12.1 A1 – Pentane, Isoprene, Ethanol, Acetone Integrity Tests

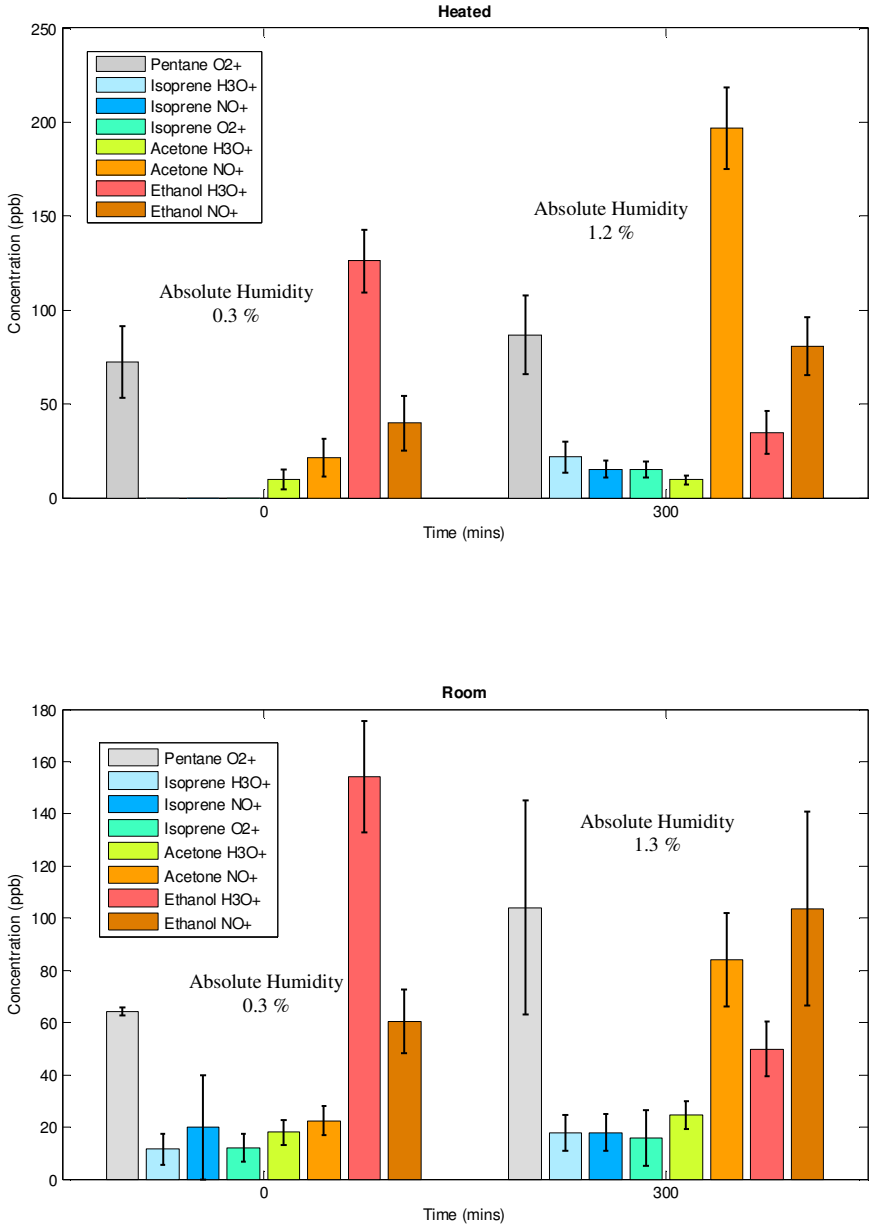


Figure A1.1: Nitrogen filled permeation test bag tested at time 0 and 360 min for both storage temperatures including the 1.96 standard deviations

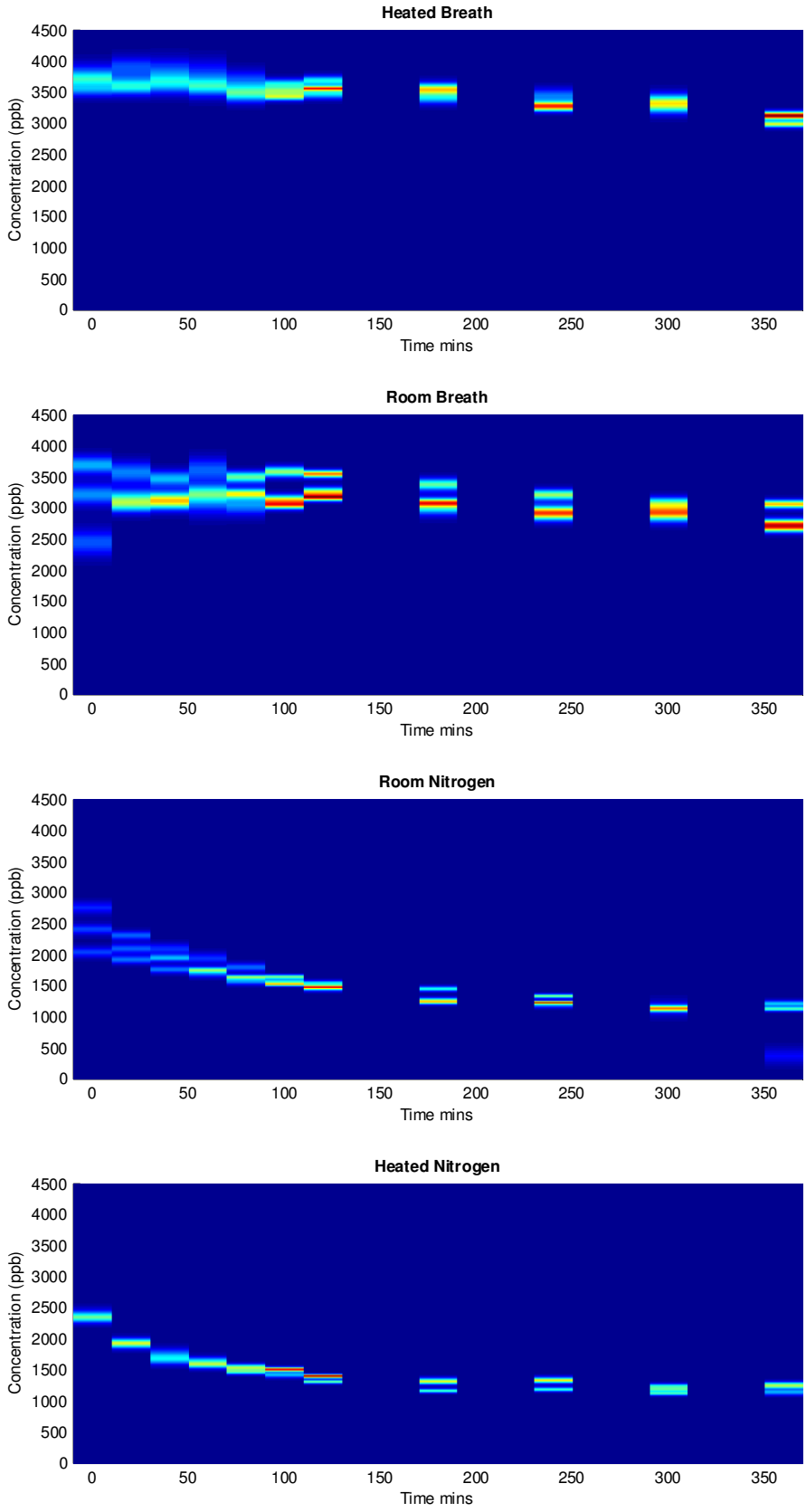


Figure A1.2: Acetone monitored using the H_3O^+ precursor in 1L bags

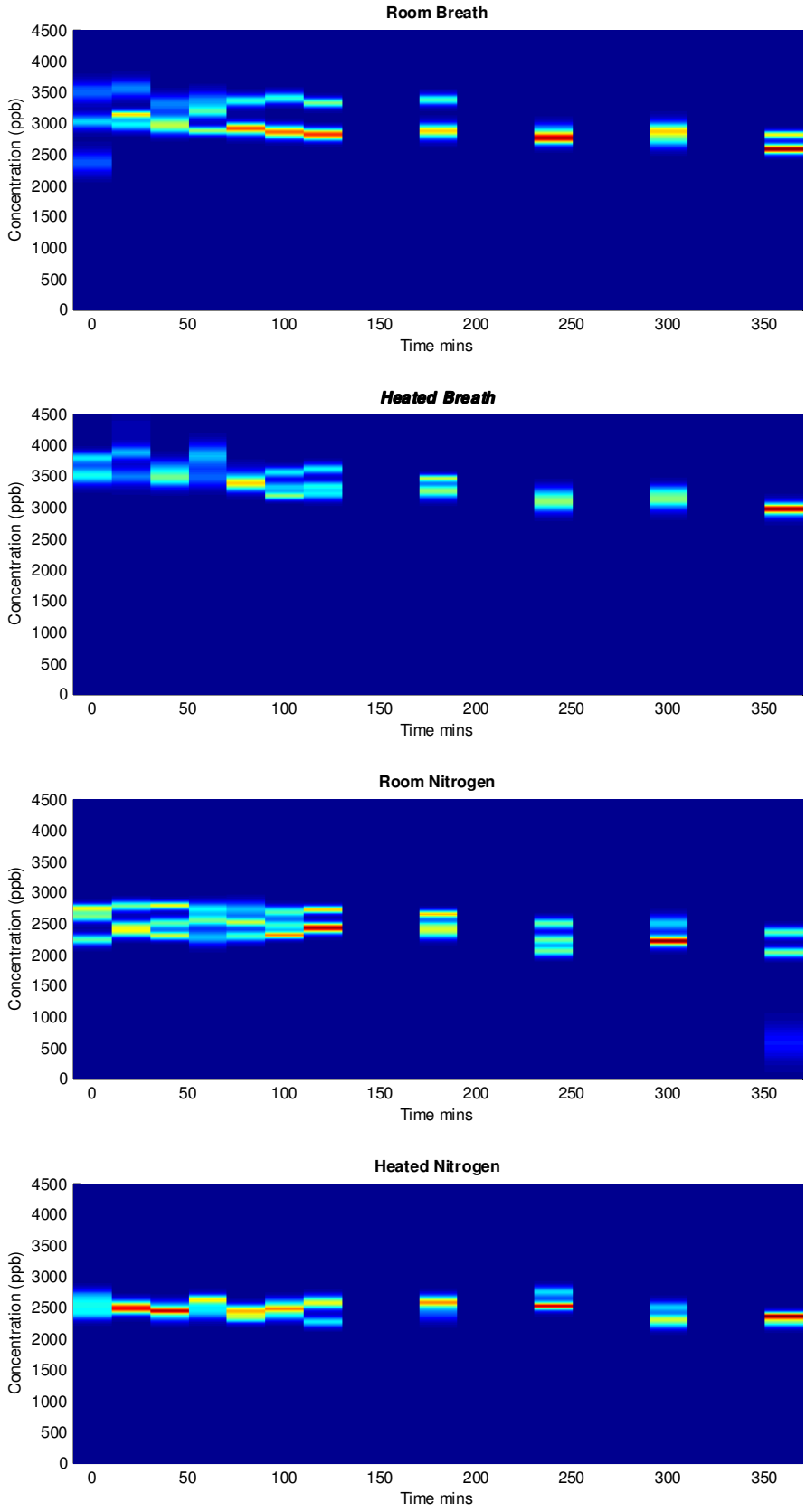


Figure A1.3: Acetone monitored using the NO^+ precursor in 1L bags

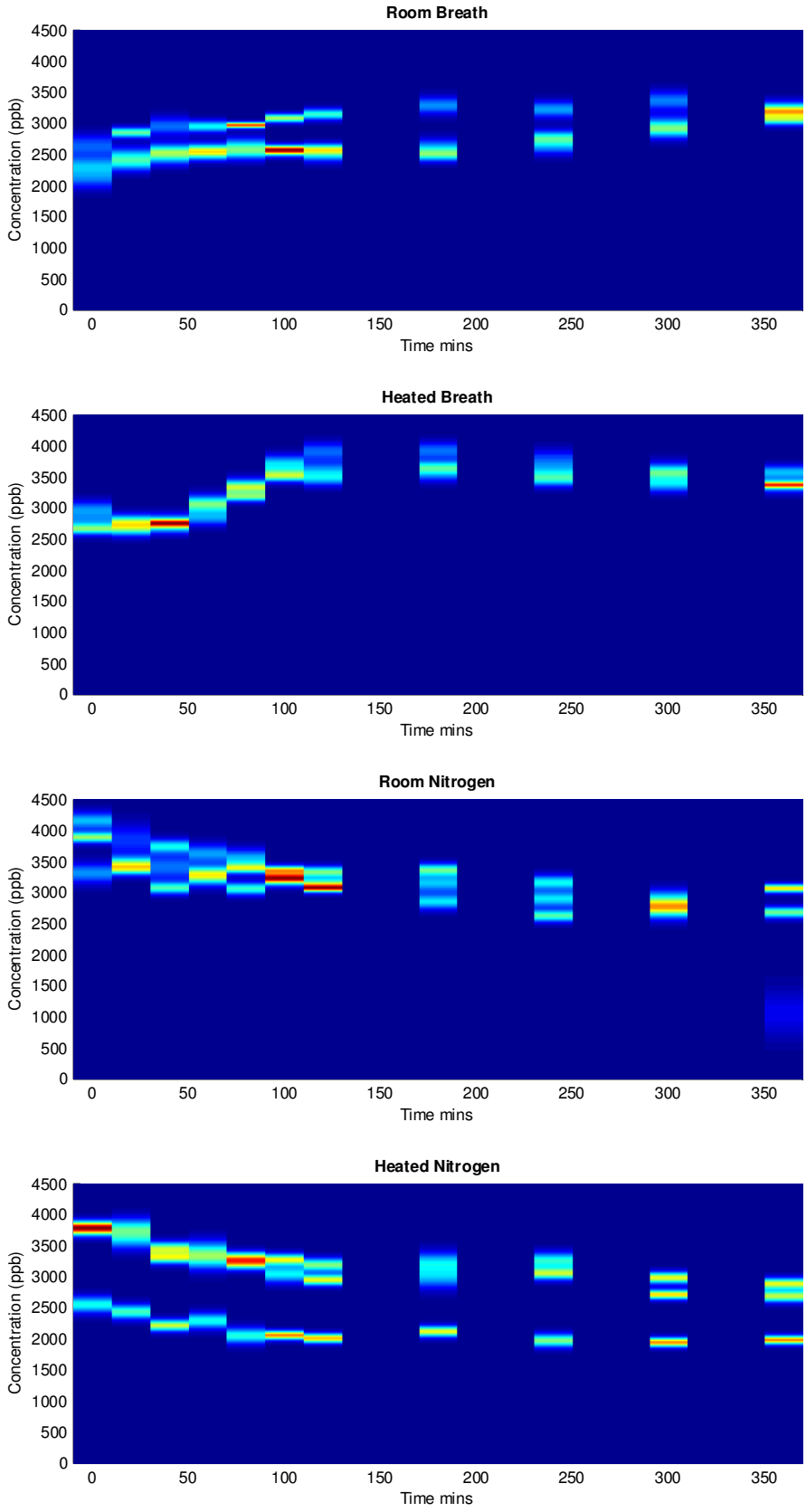


Figure A1.4: Ethanol monitored using the NO^+ precursor in 1L bags

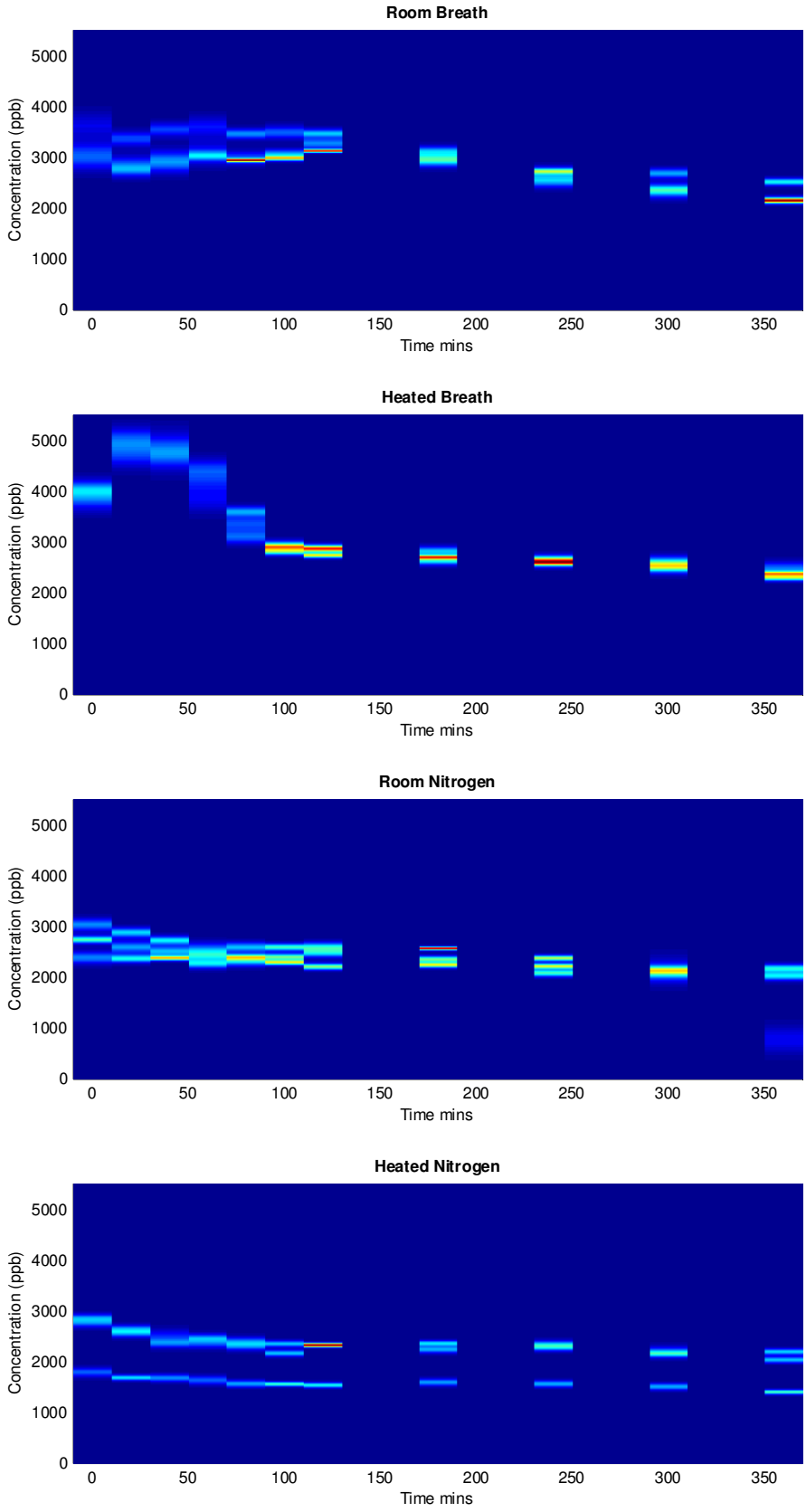


Figure A1.5: Ethanol monitored using the H_3O^+ precursor in 1L bags

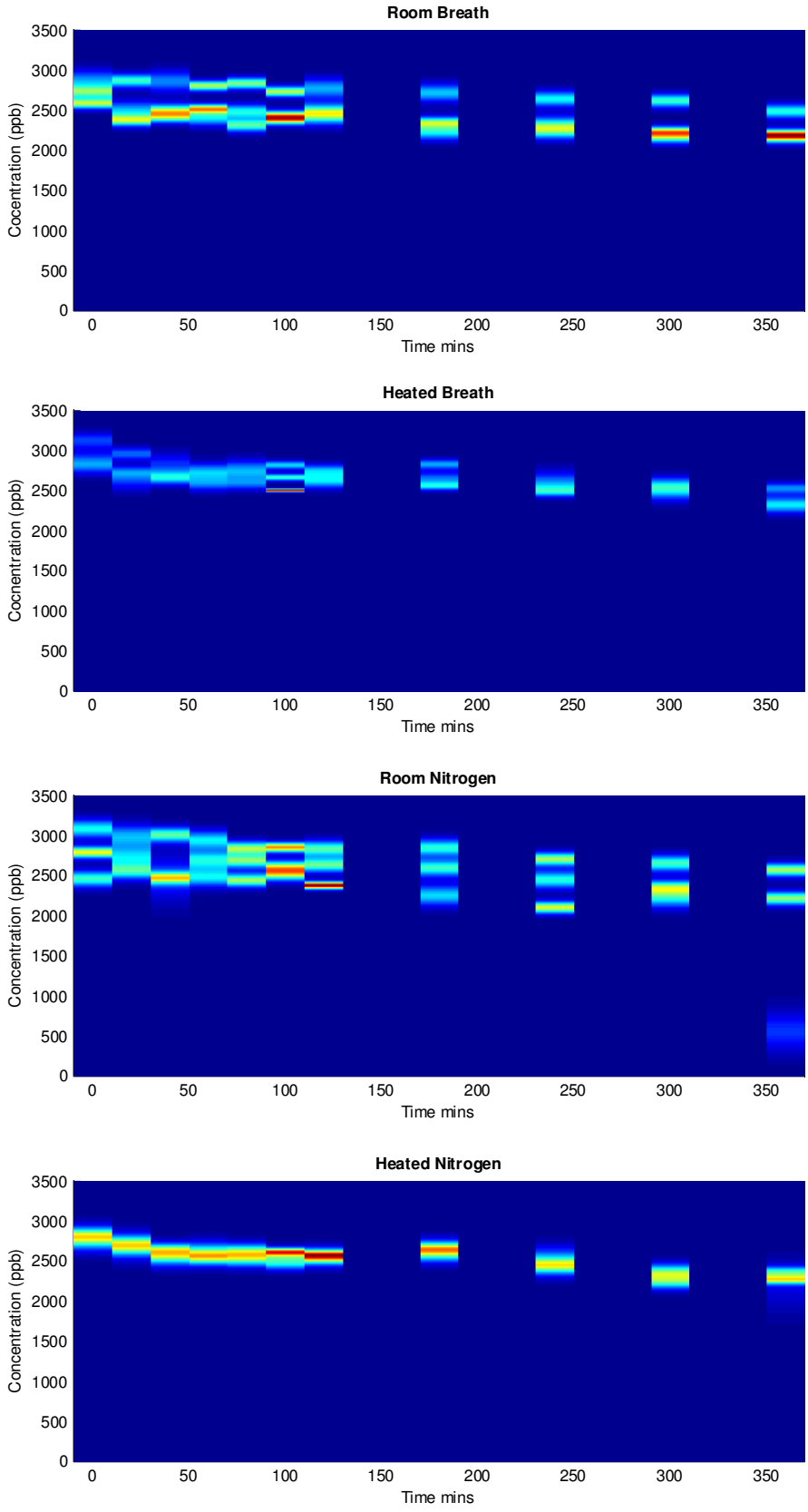


Figure A1.6: Isoprene monitored using the NO^+ precursor in 1L bags

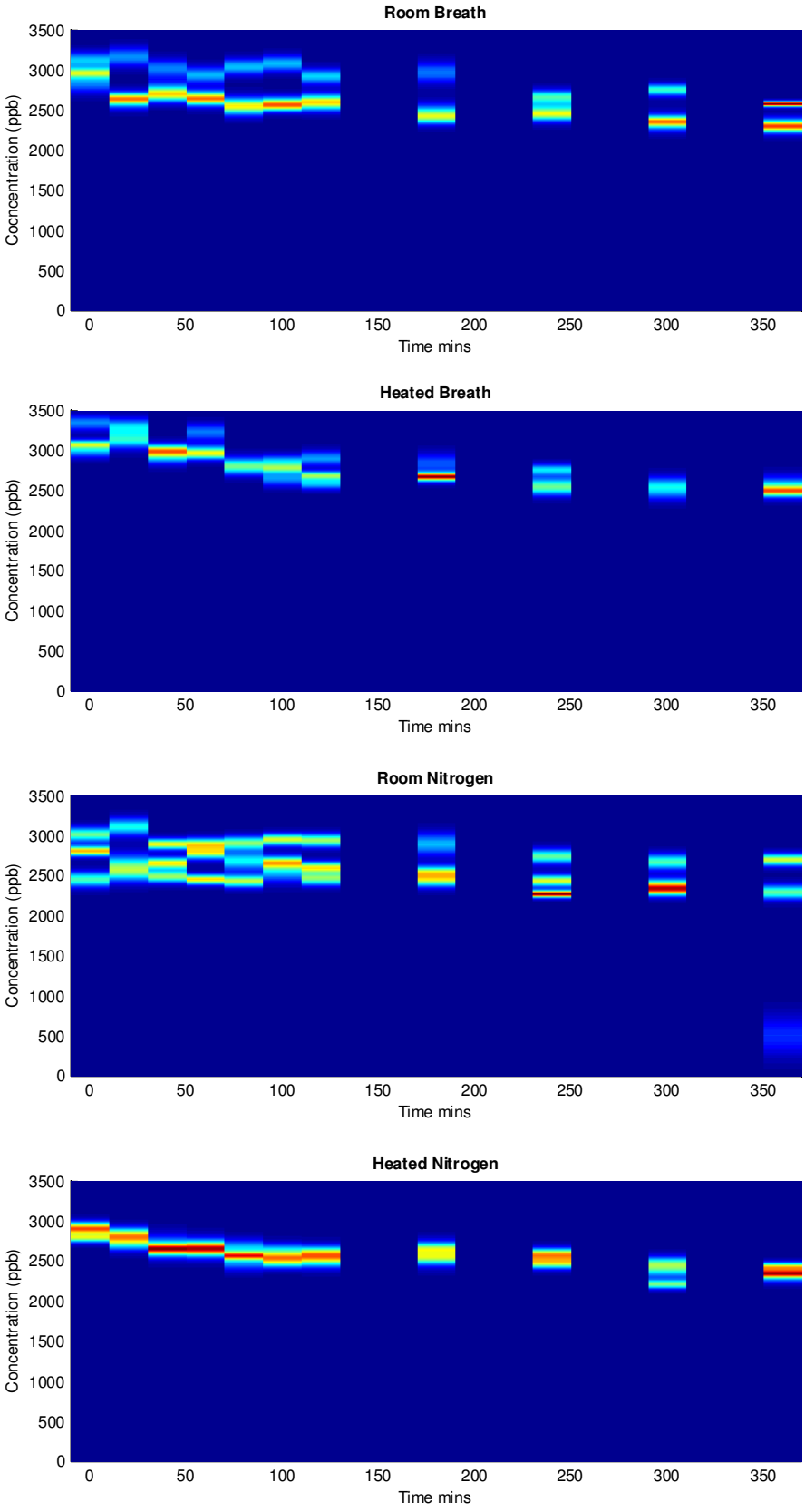


Figure A1.7: Isoprene monitored using the O_2^+ precursor in 1L bags

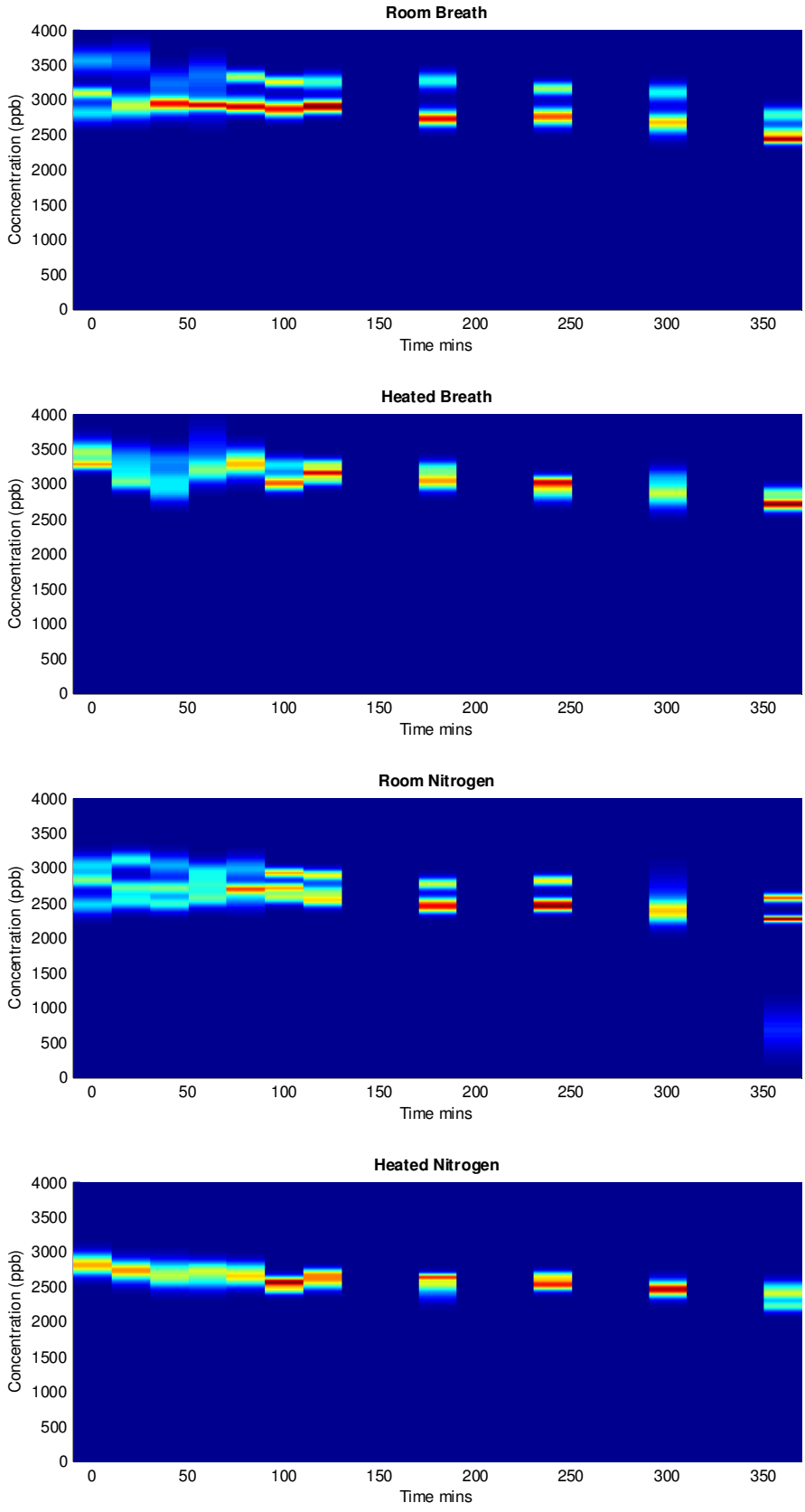


Figure A1.8: Isoprene monitored using the H_3O^+ precursor in 1L bags

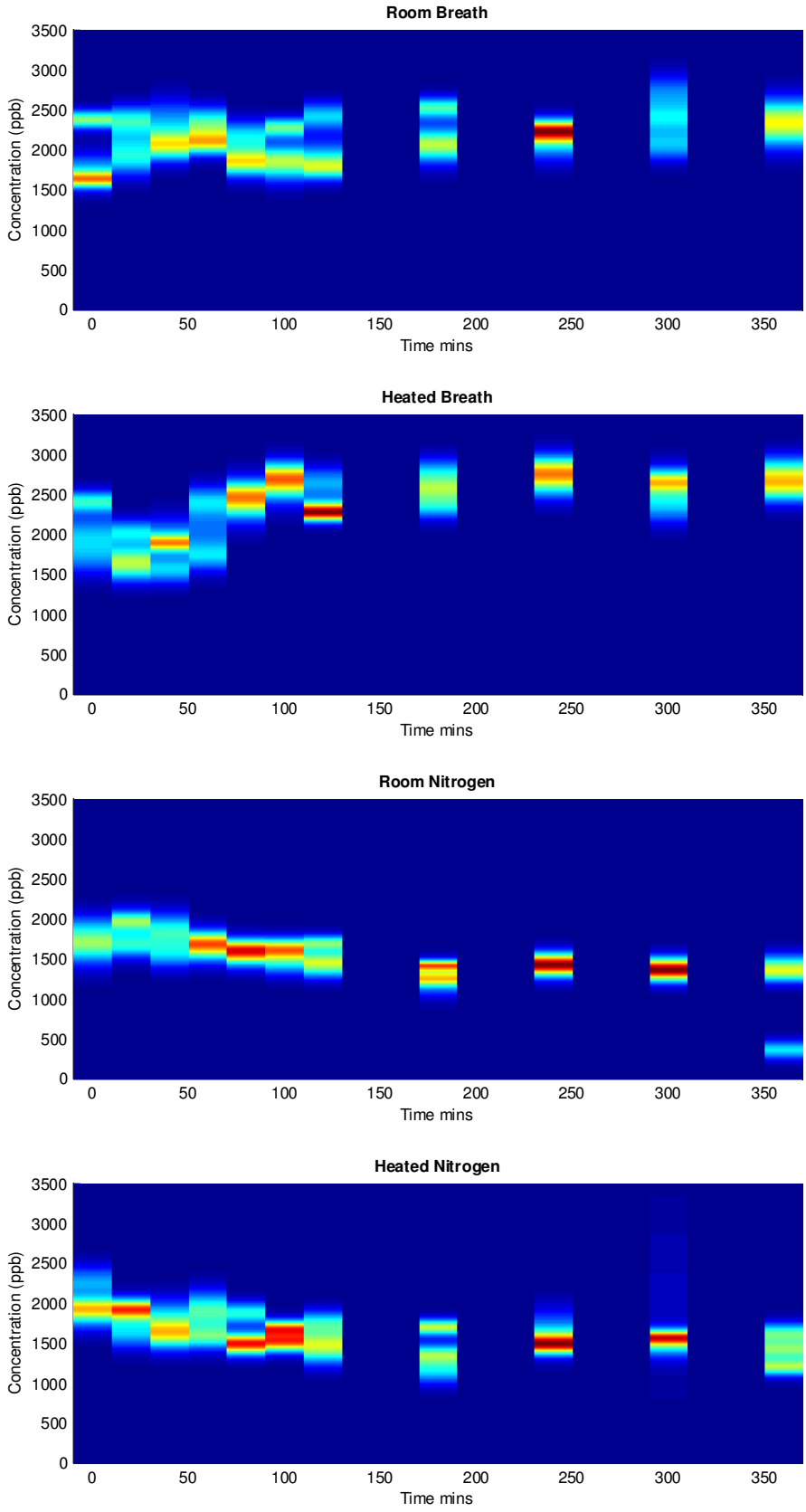


Figure A1.9: Pentane monitored using the O_2^+ precursor in 1L bags

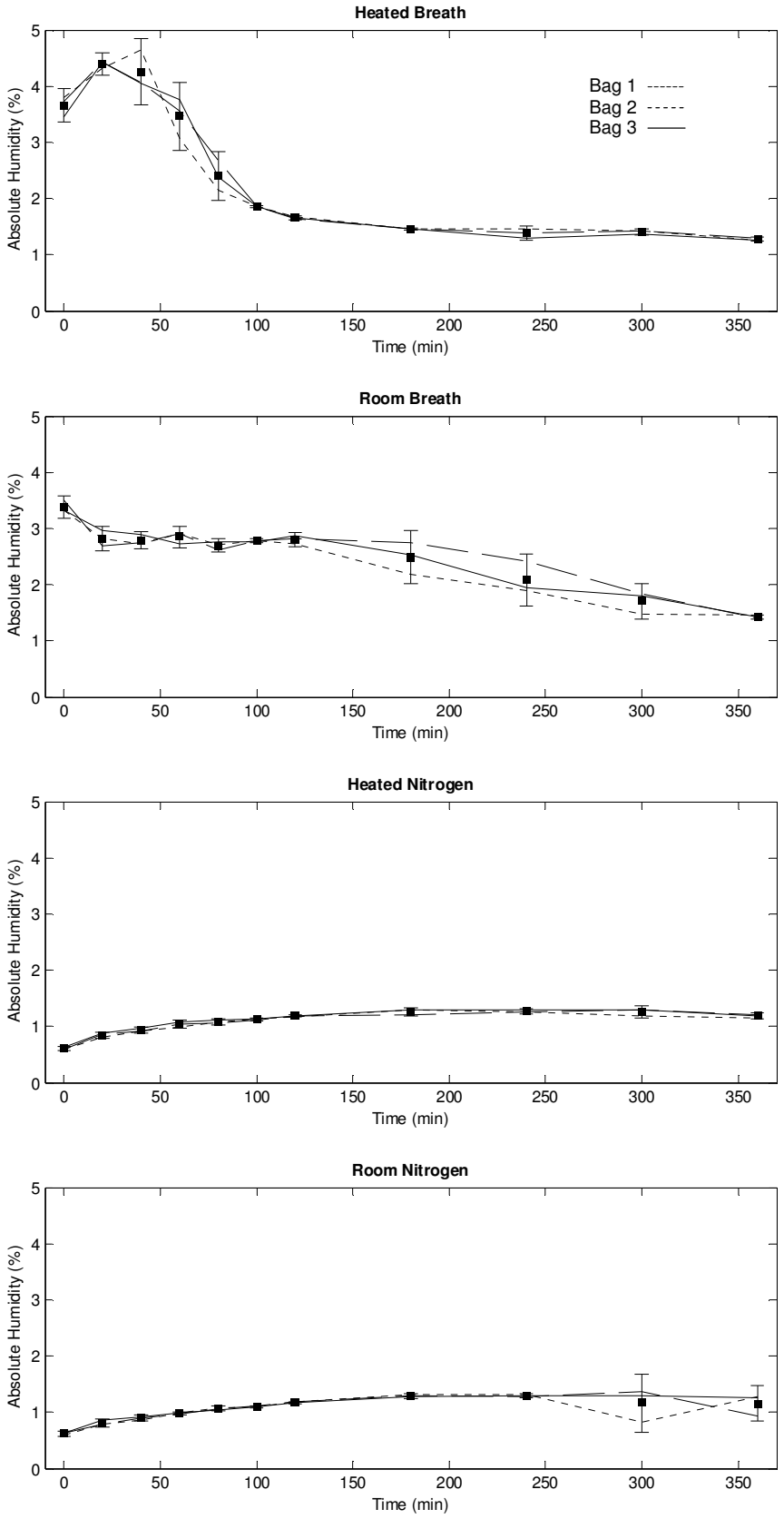


Figure A1.10: Absolute humidity in 1L bags for each substrate and storage temperature

12.2 A2 – Pentane, Isoprene, Ethanol, Acetone Storage Size Tests

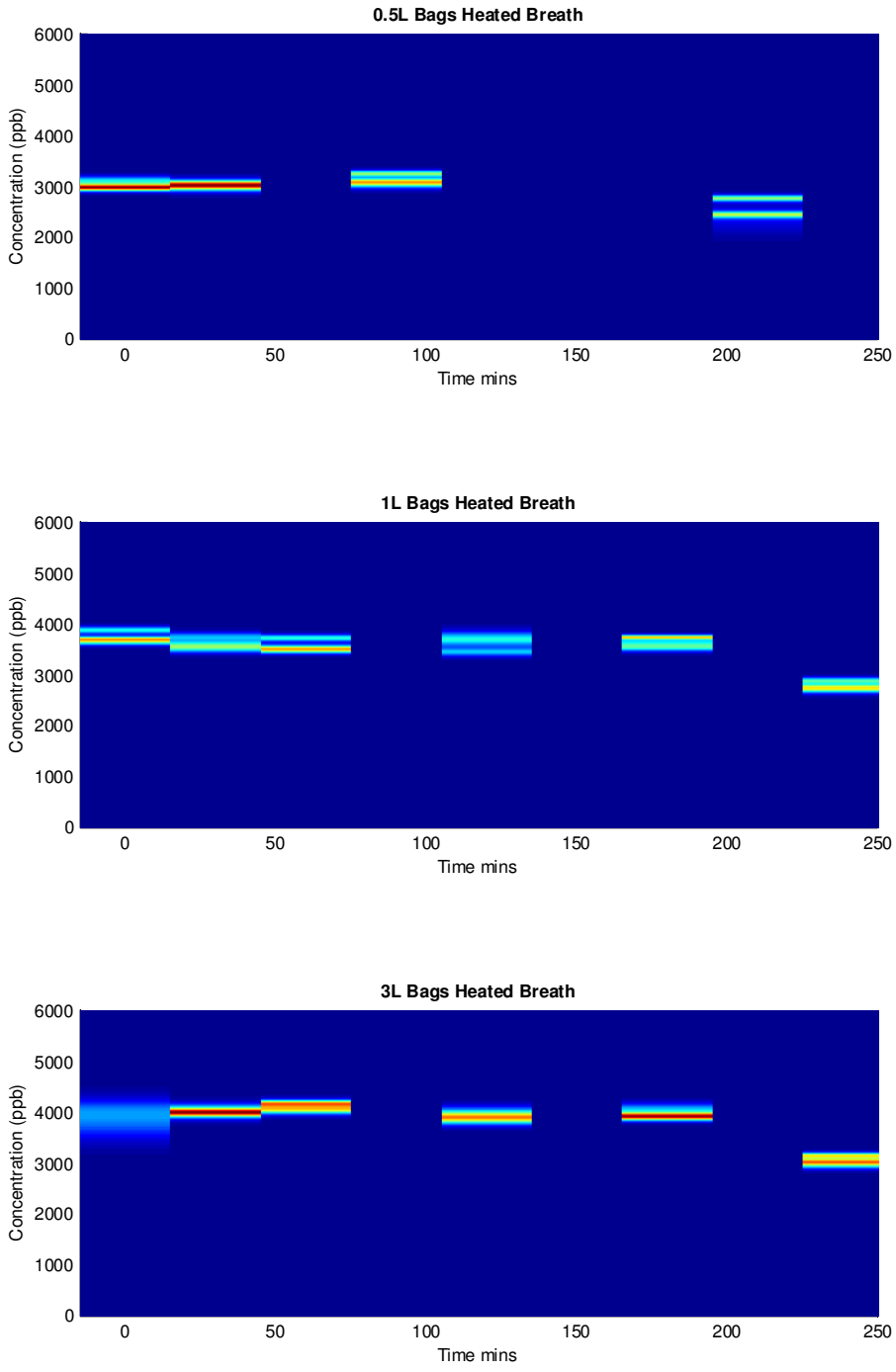


Figure A2.1: Acetone monitored using the H_3O^+ precursor in heated breath

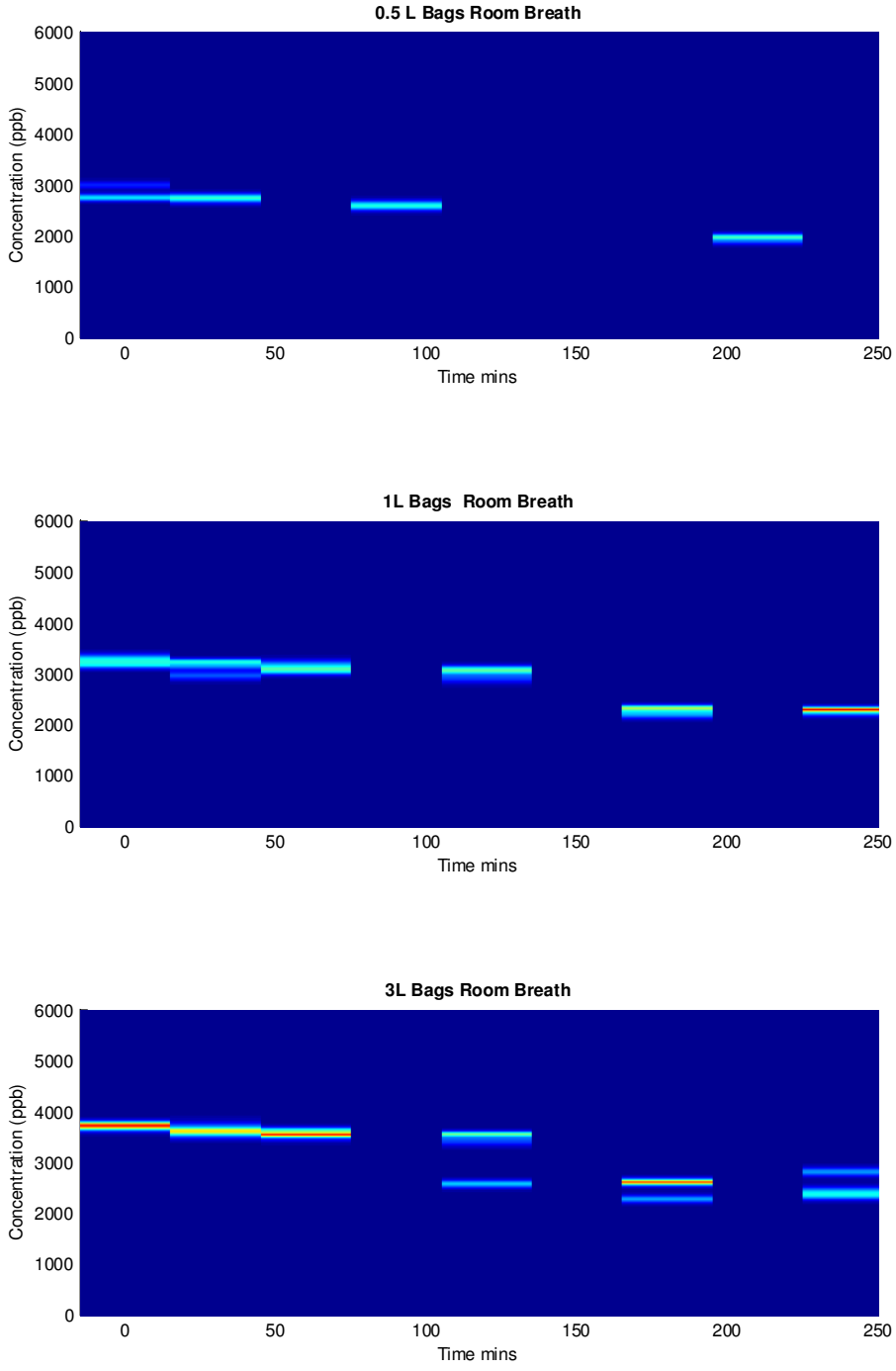


Figure A2. 2: Acetone monitored using the H_3O^+ precursor in room breath

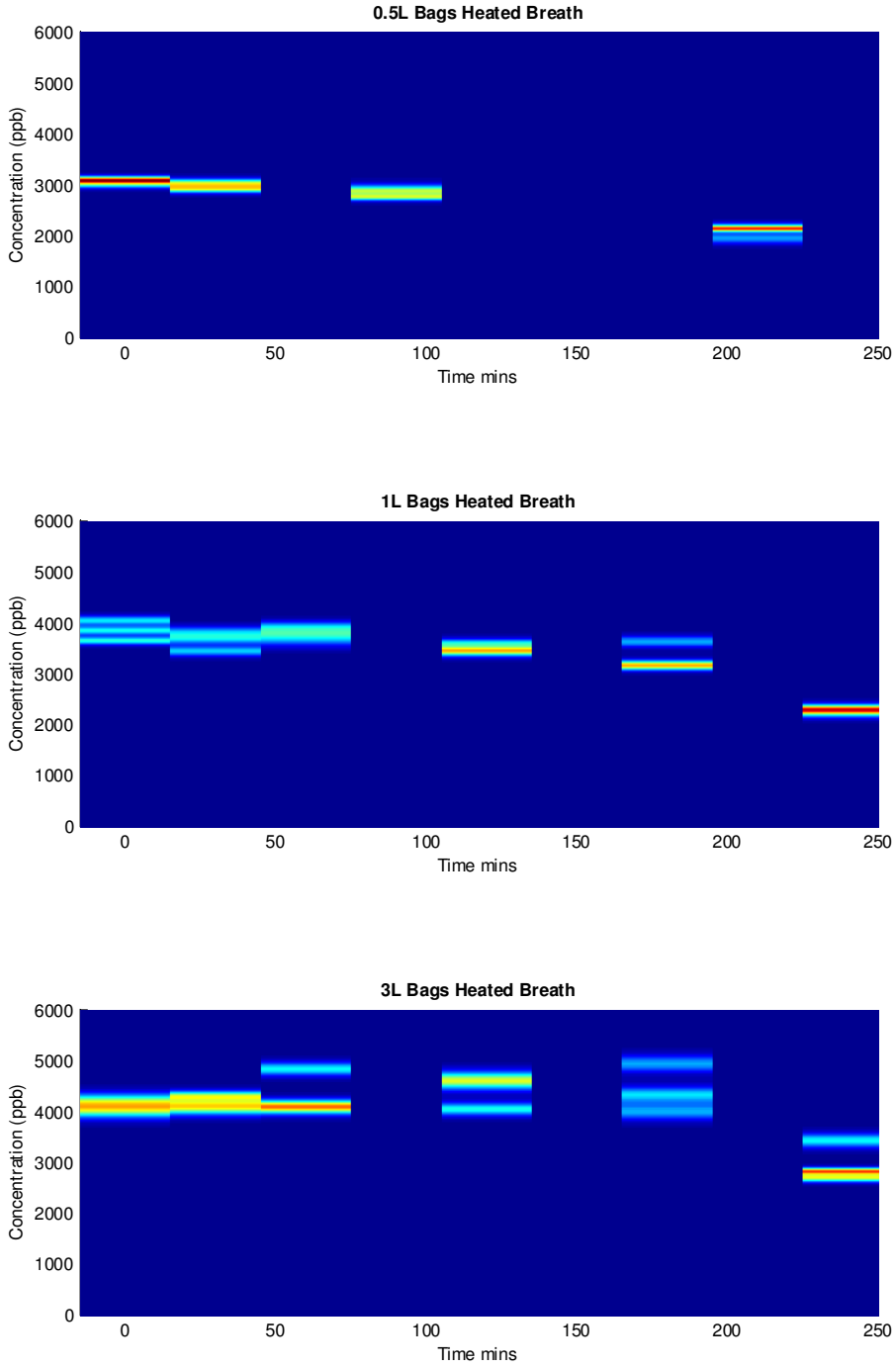


Figure A2. 3: Acetone monitored using the NO^+ precursor in heated breath

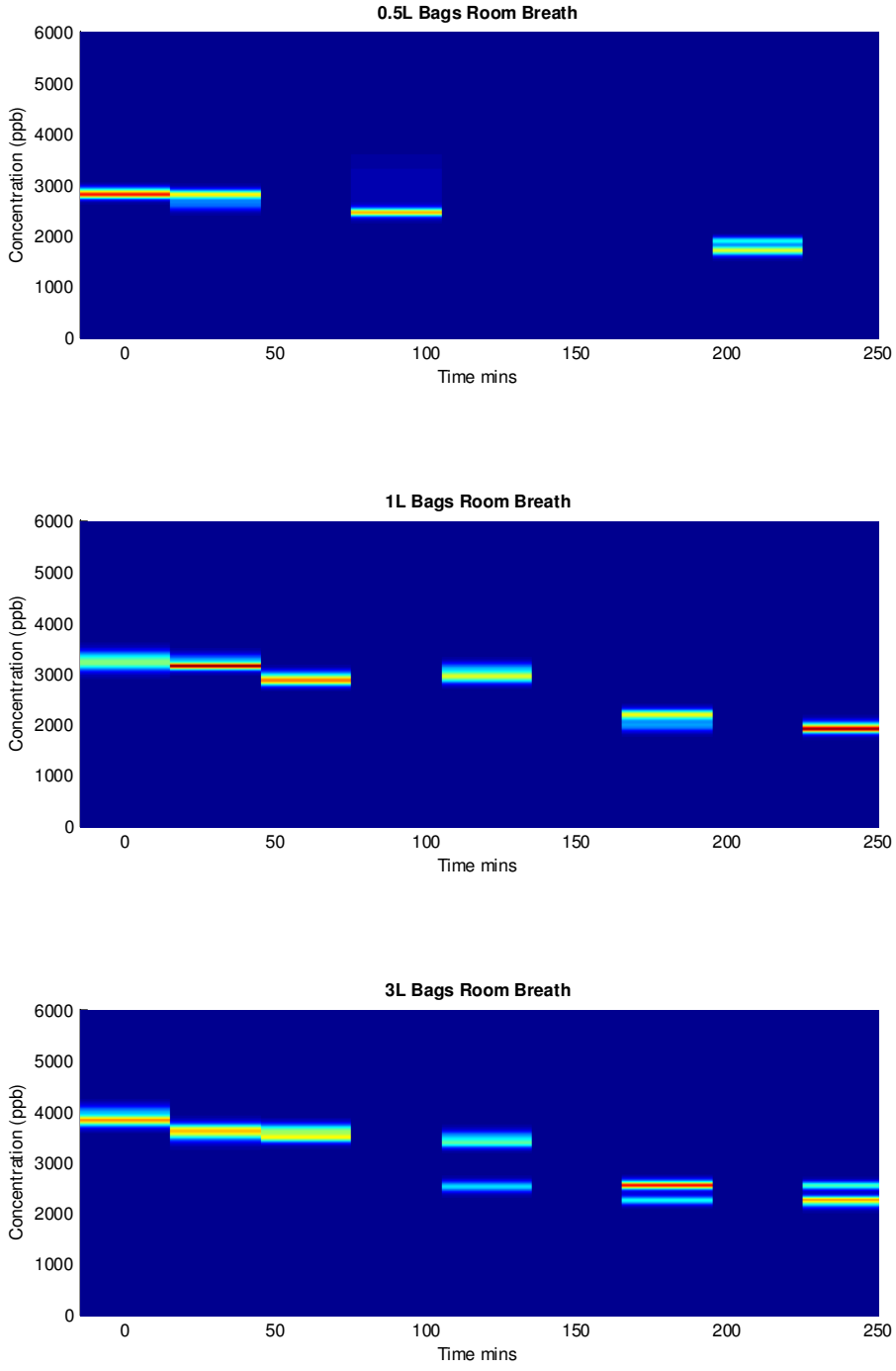


Figure A2. 4: Acetone monitored using the NO^+ precursor in room breath

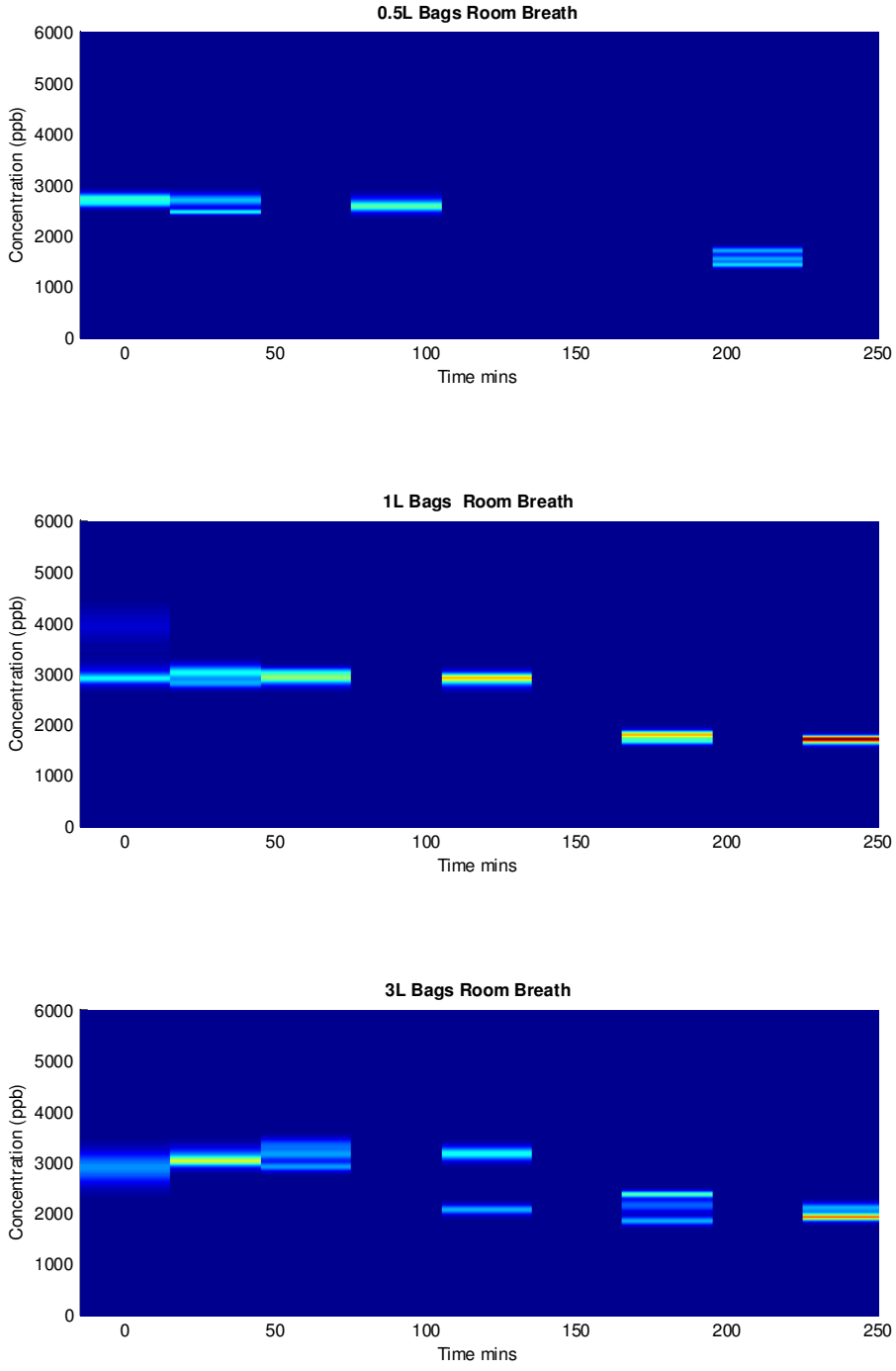


Figure A2. 5: Ethanol monitored using the H_3O^+ precursor in room breath

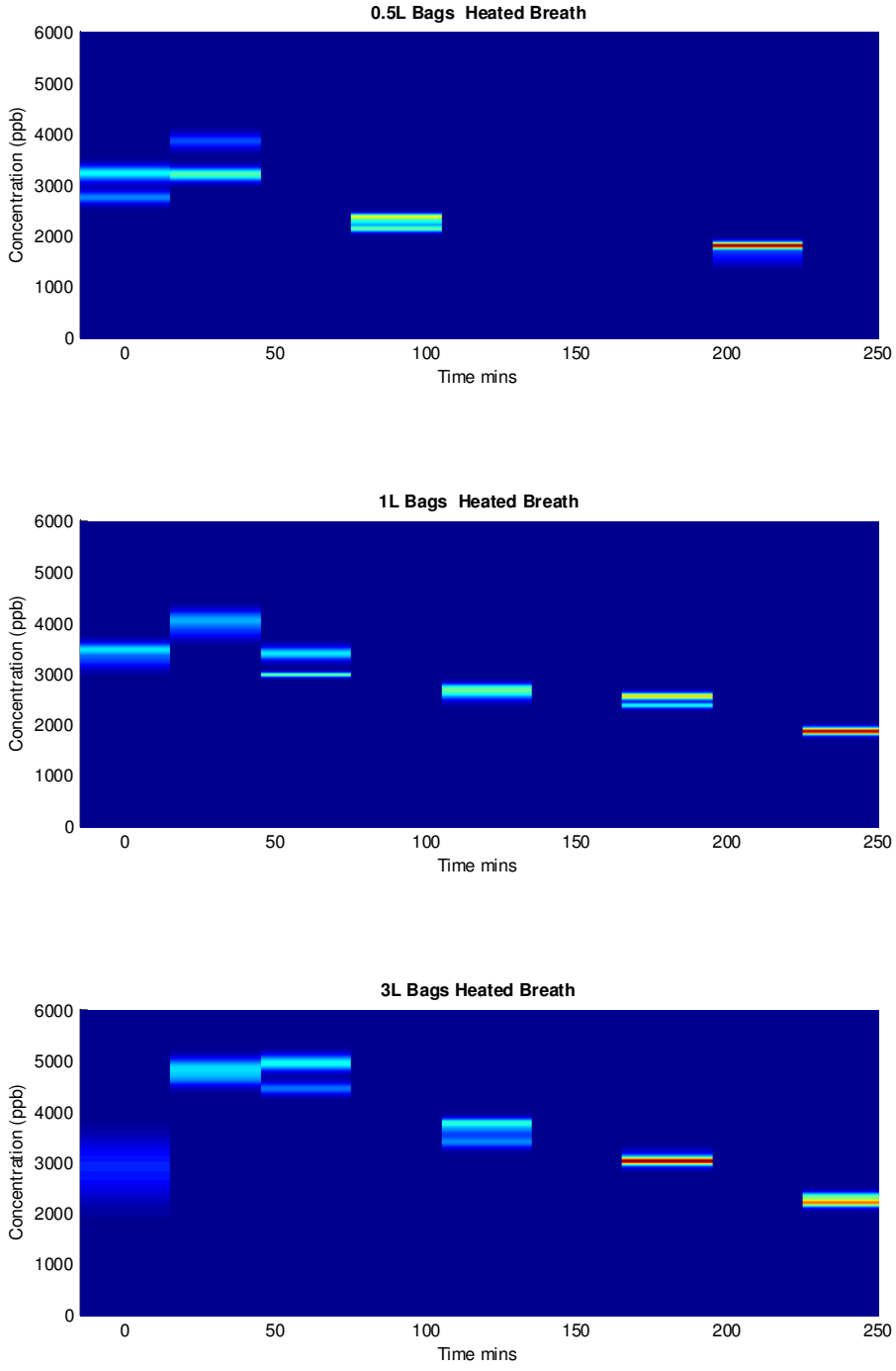


Figure A2. 6: Ethanol monitored using the H_3O^+ precursor in heated breath

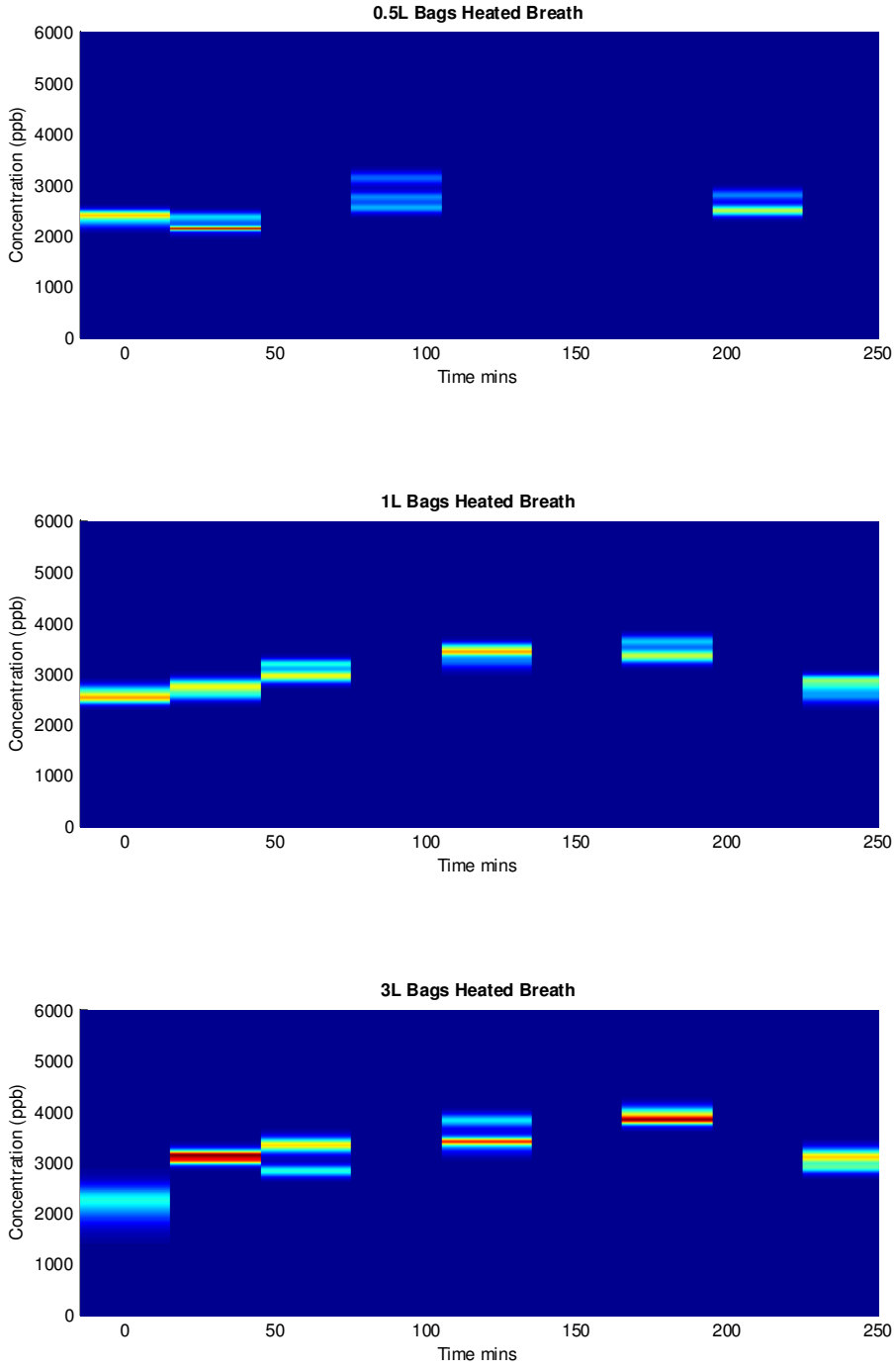


Figure A2. 7: Ethanol monitored using the NO^+ precursor in heated breath

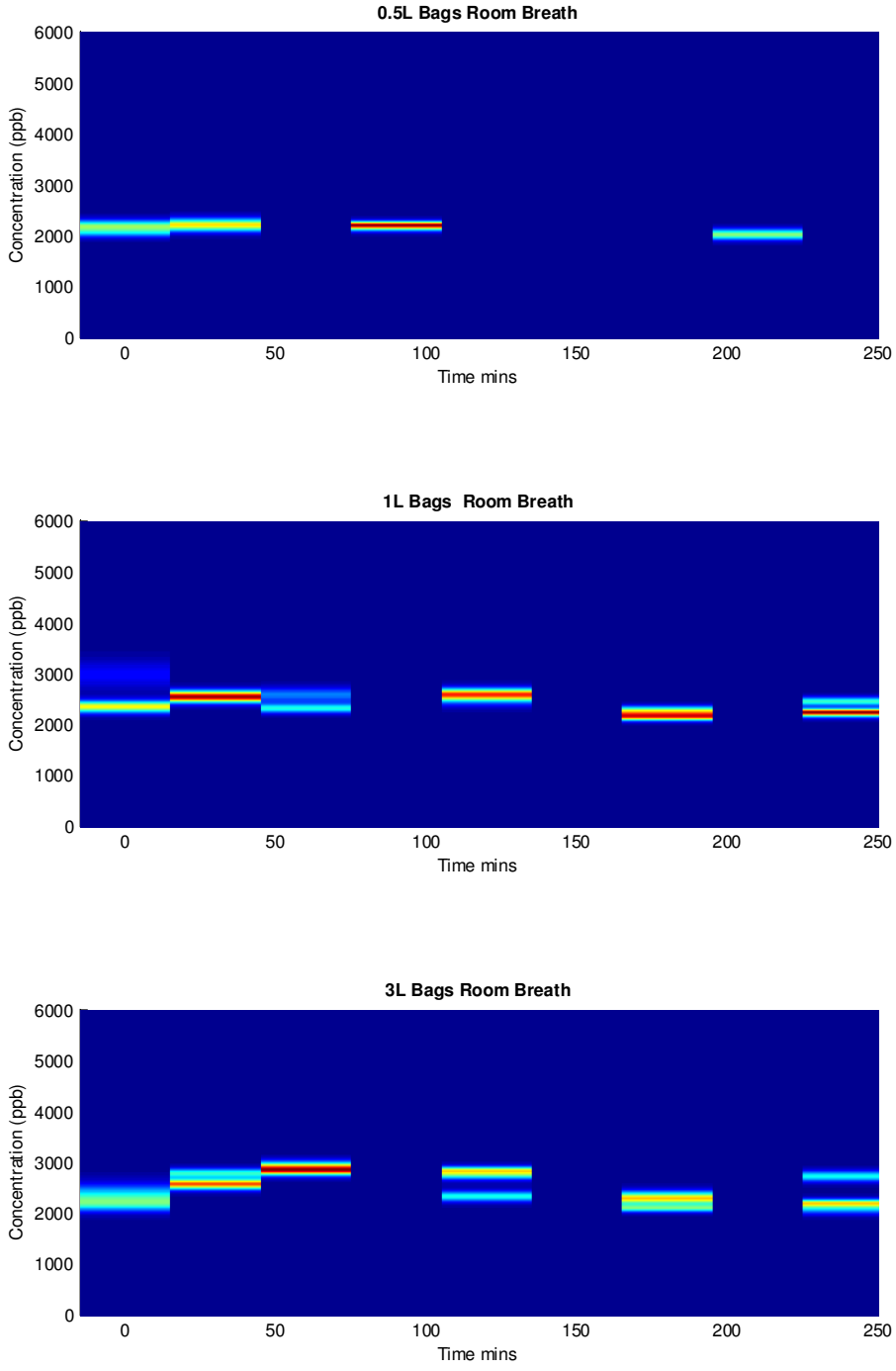


Figure A2. 8: Ethanol monitored using the NO^+ precursor in room breath

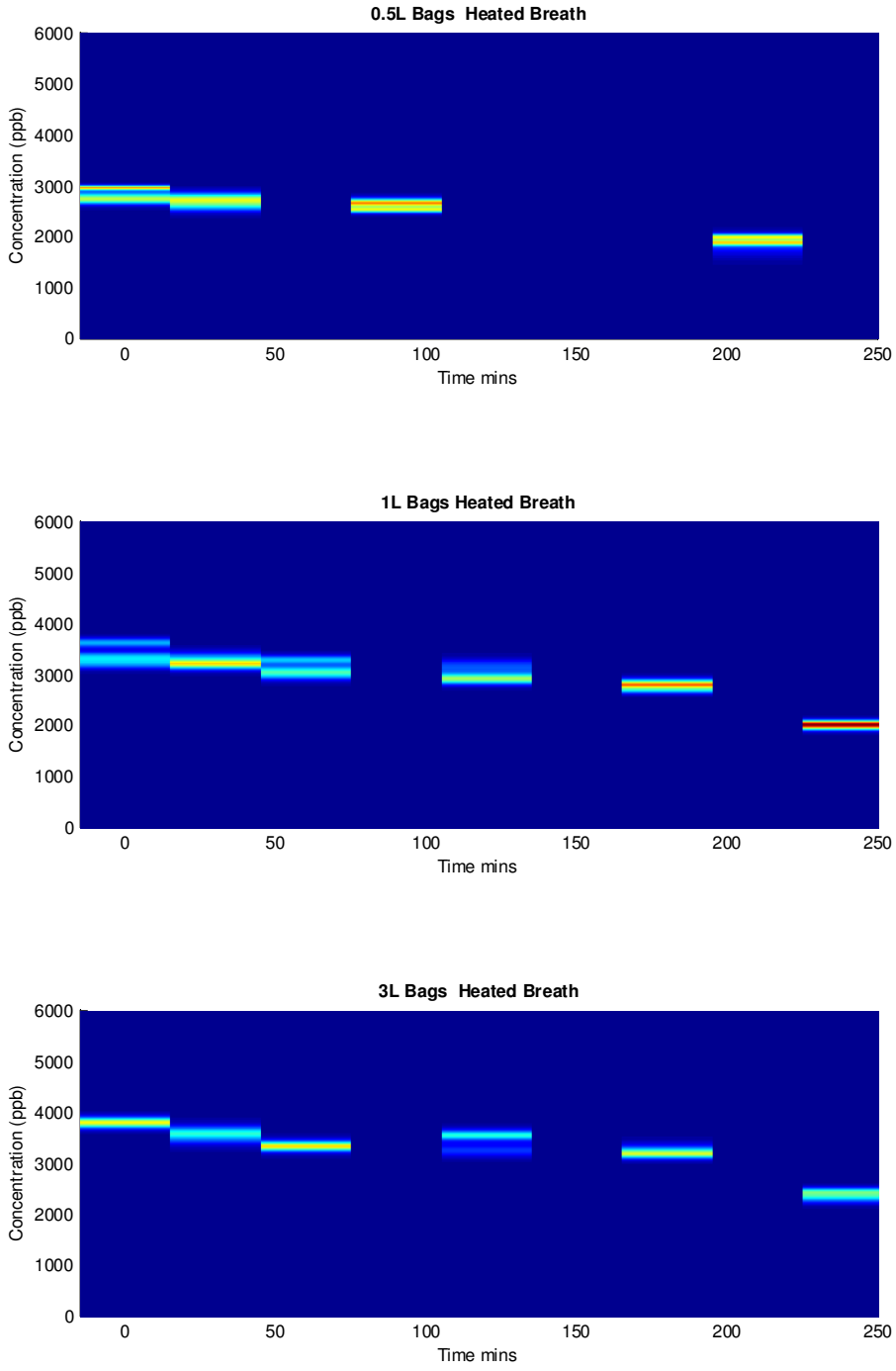


Figure A2. 9: Isoprene monitored using the H_3O^+ precursor in heated breath

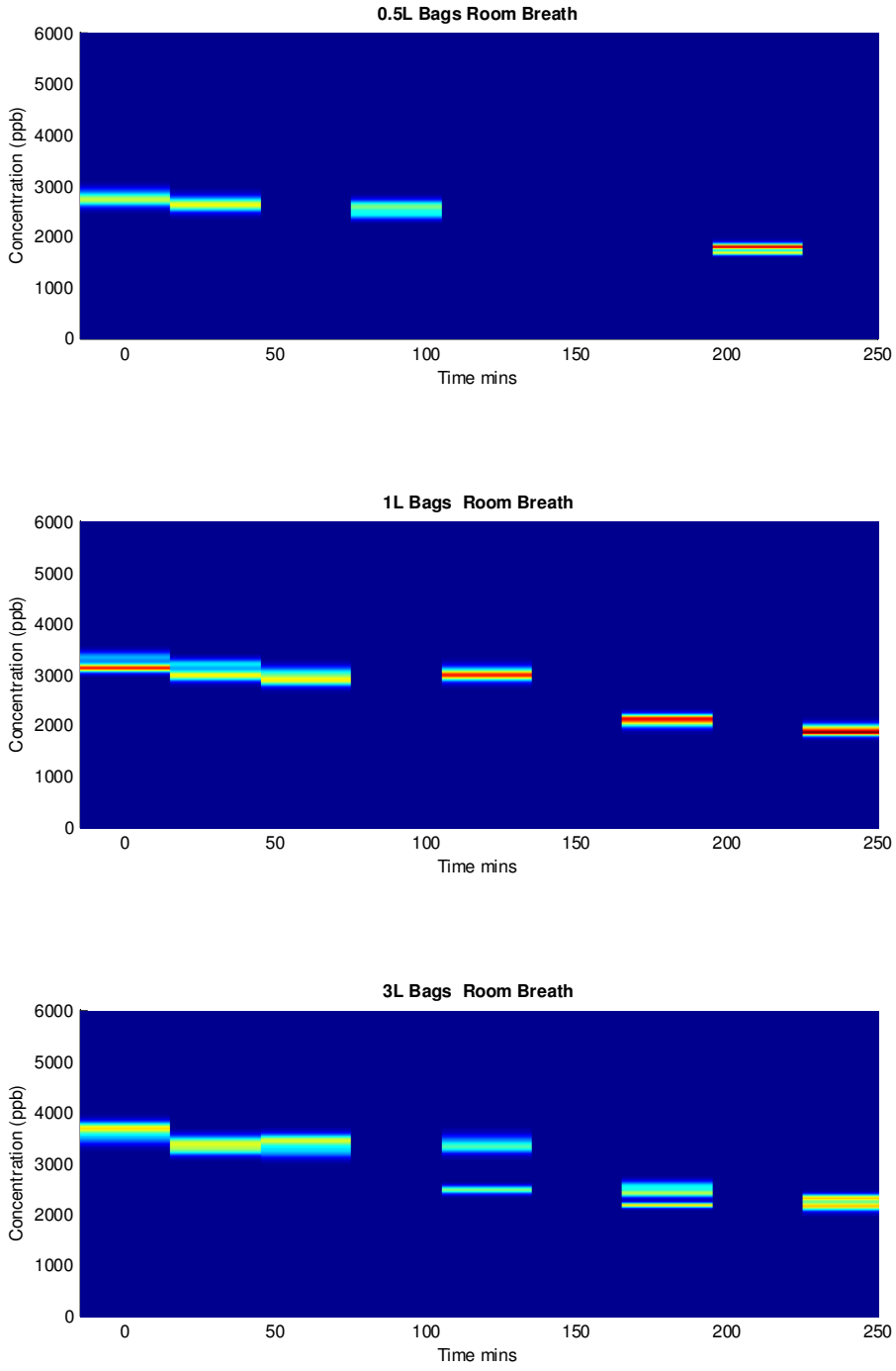


Figure A2. 10: Isoprene monitored using the H_3O^+ precursor in room breath

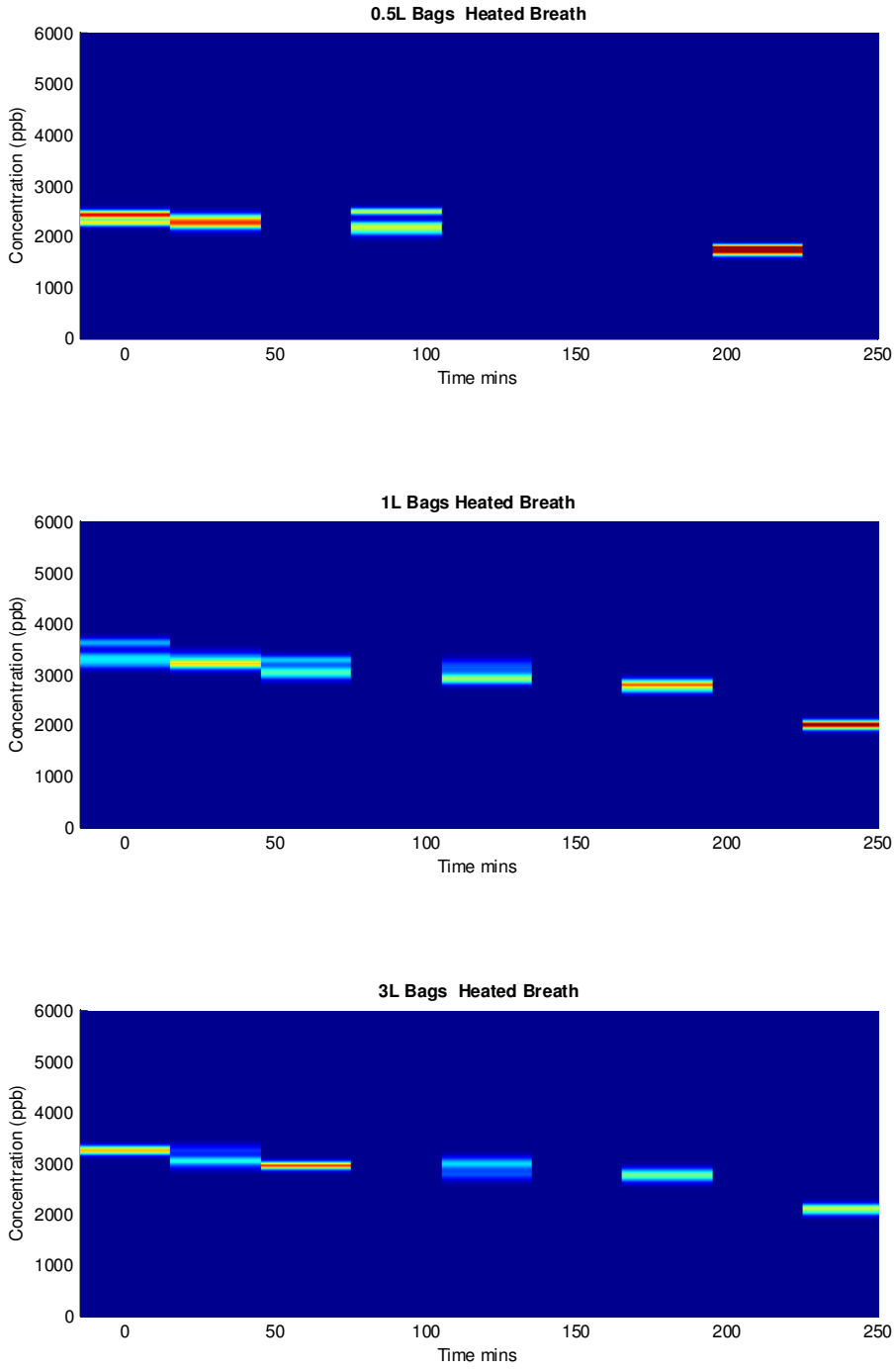


Figure A2. 11: Isoprene monitored using the NO^+ precursor in heated breath

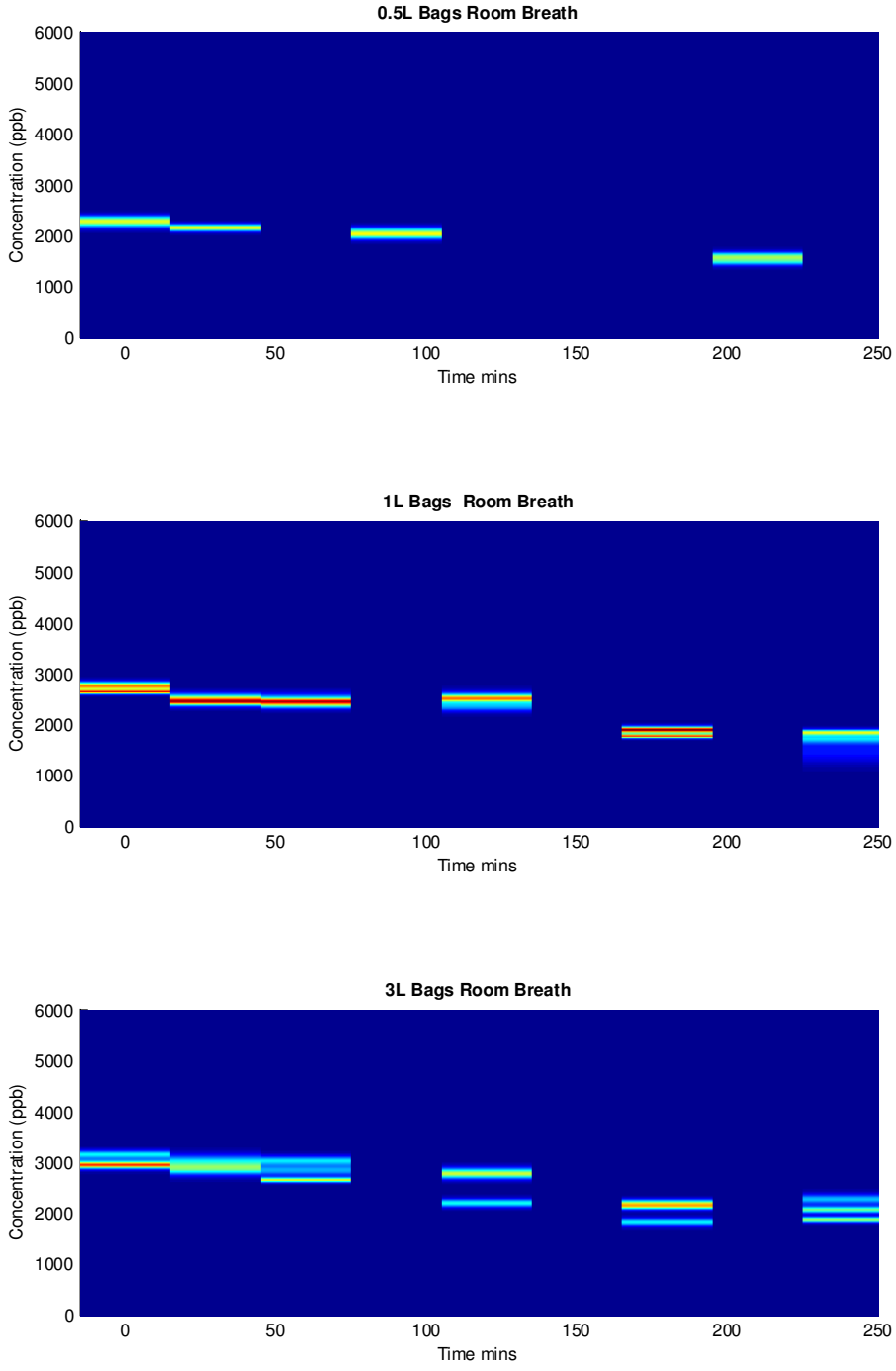


Figure A2. 12: Isoprene monitored using the NO^+ precursor in room breath

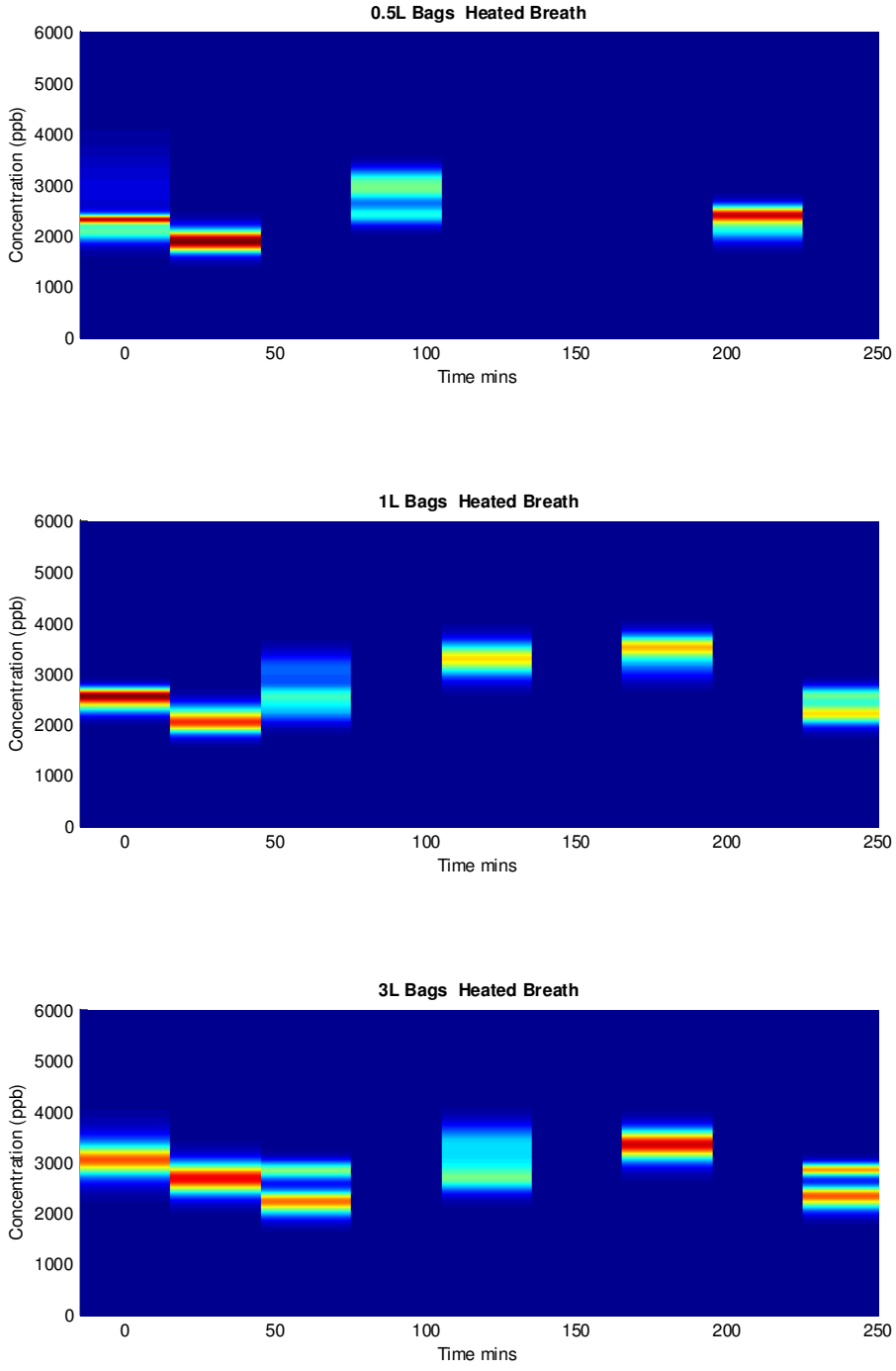


Figure A2. 13: Pentane monitored using the O_2^+ precursor in heated breath

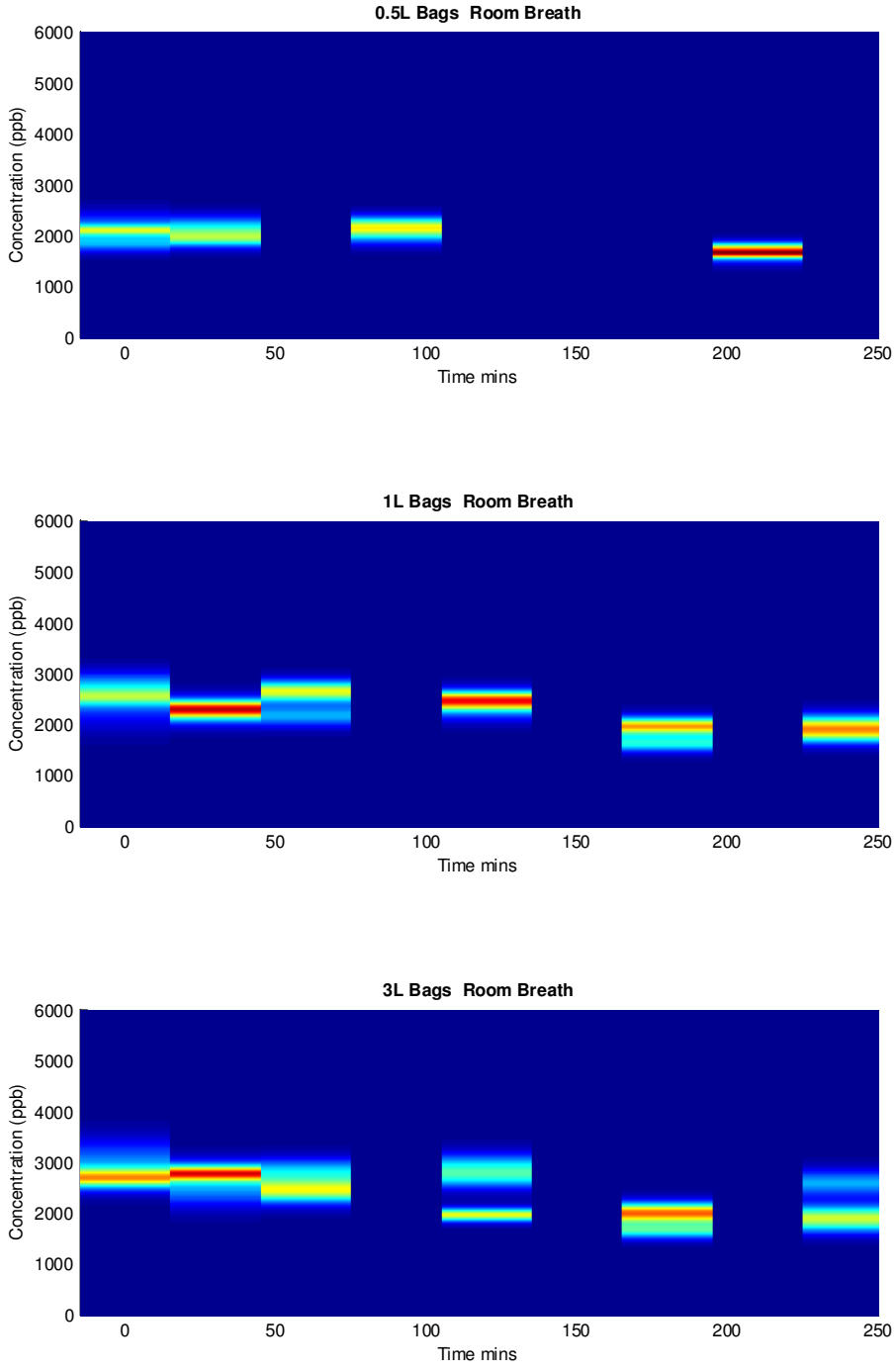


Figure A2. 14: Pentane monitored using the O_2^+ precursor in room breath

12.3 A3 – Ammonia Integrity Tests

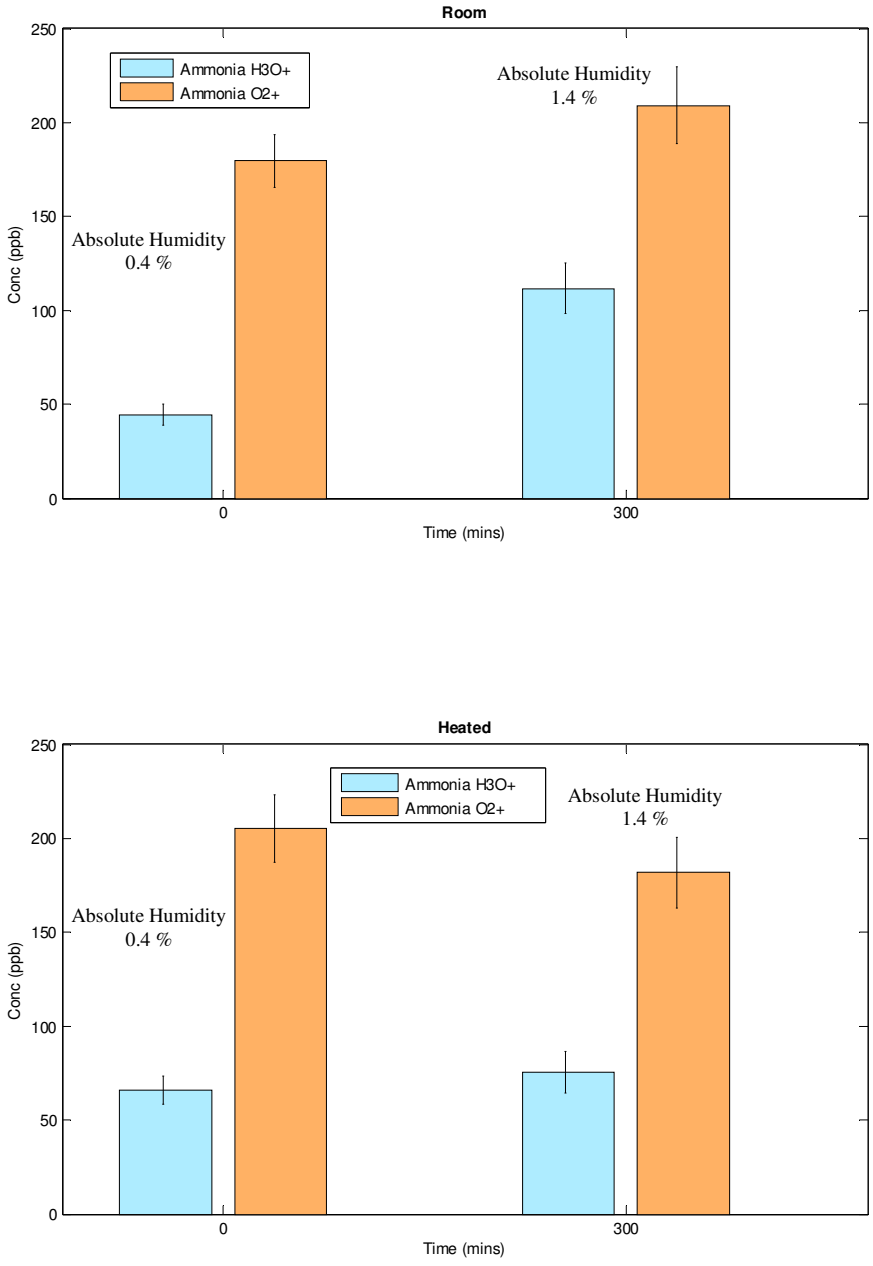


Figure A3. 1: Nitrogen filled permeation test bag tested at time 0 and 360 min for both storage temperatures including the 1.96 standard deviations

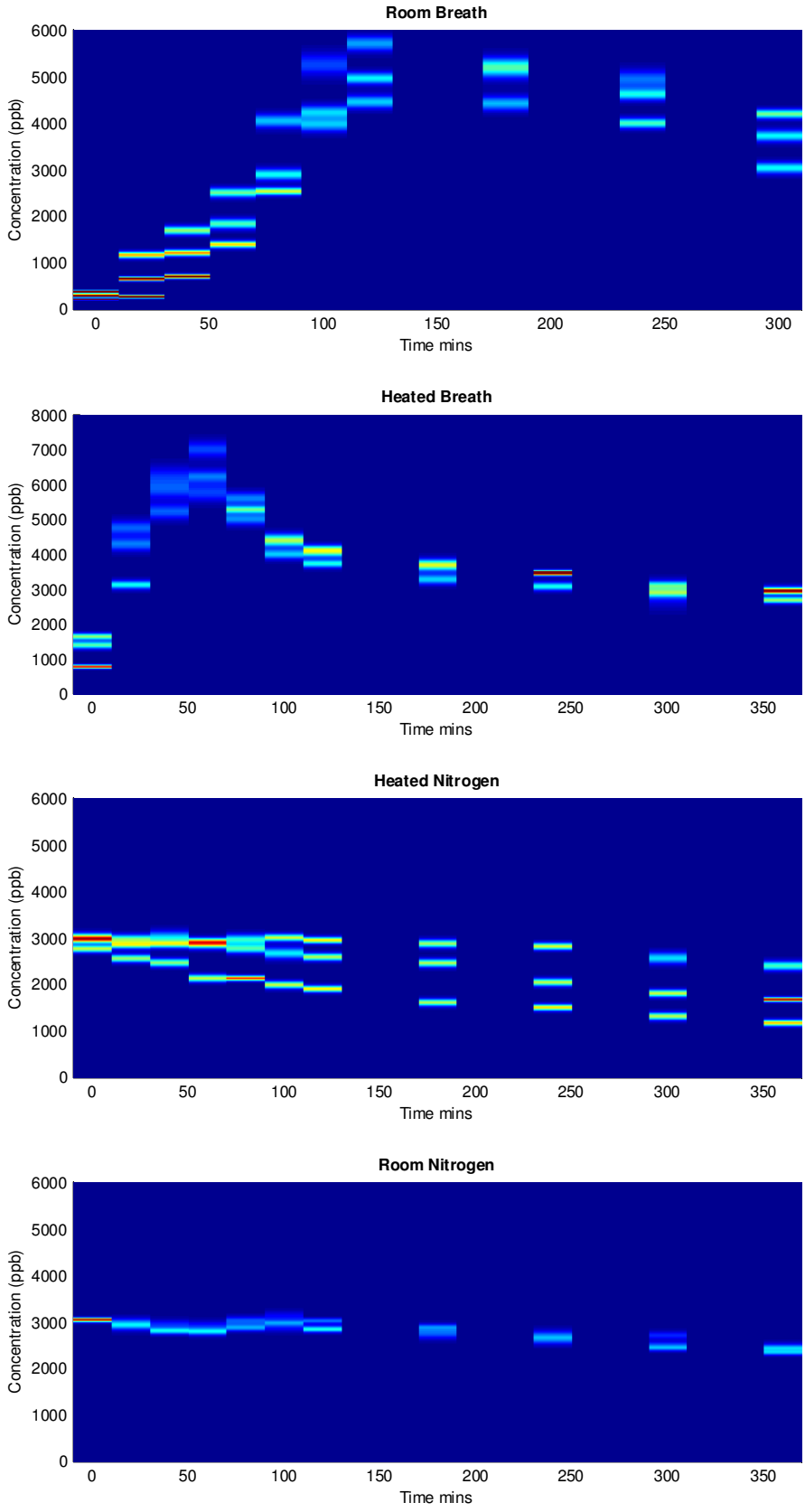


Figure A3. 2: Ammonia monitored using the H_3O^+ precursor in 1L bags test set 1

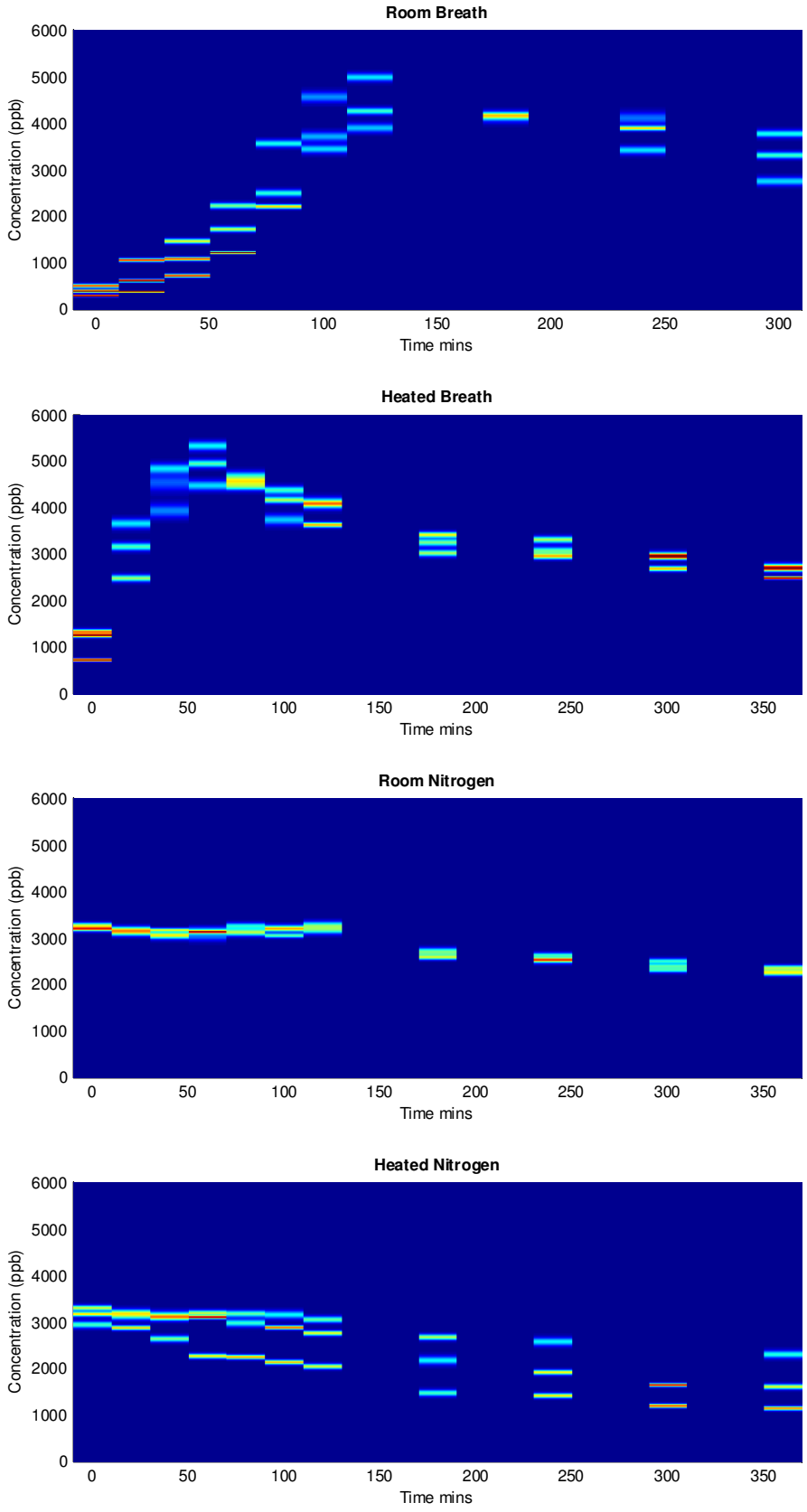


Figure A3. 3: Ammonia monitored using the O_2^+ precursor in 1L bags bags test set 1

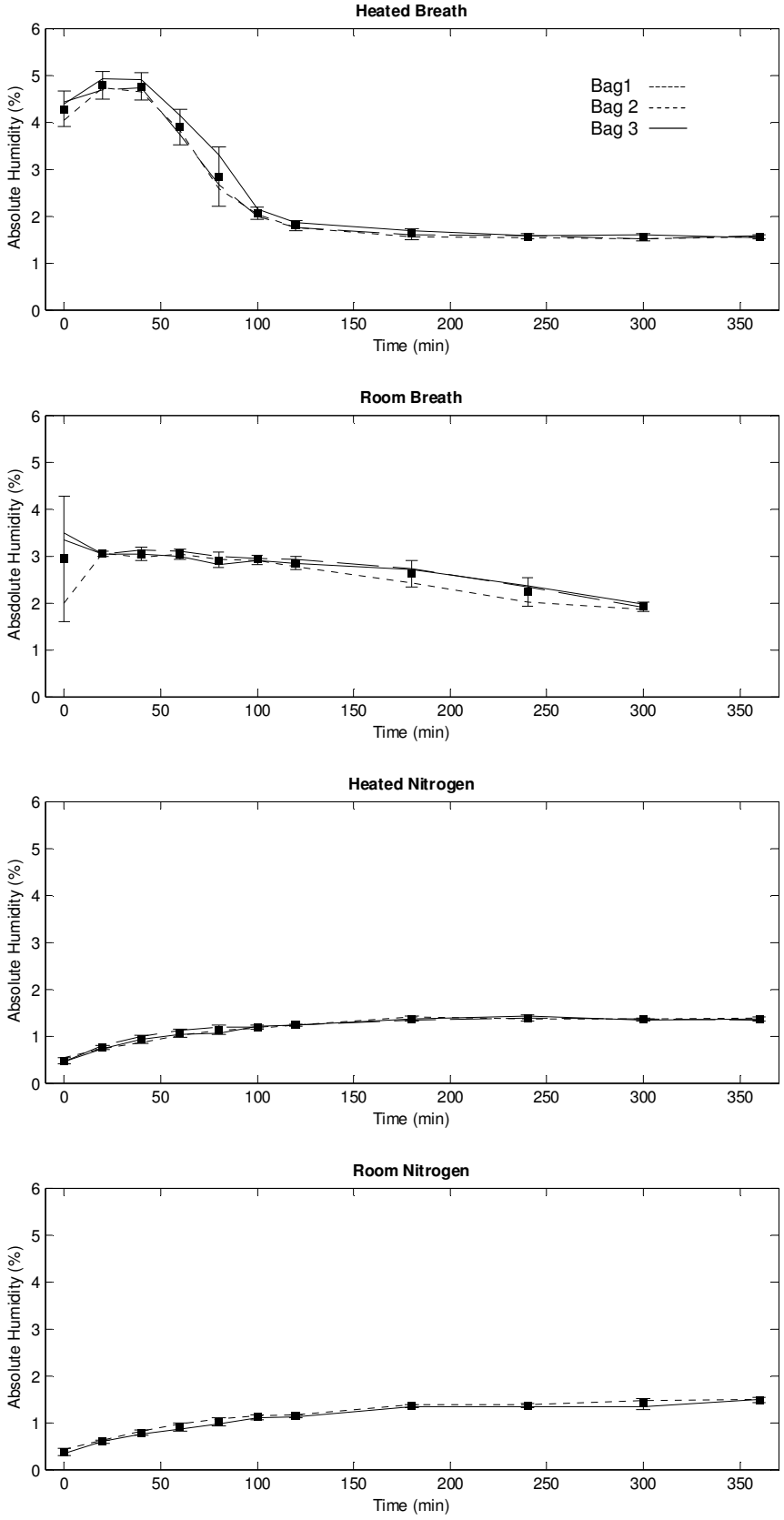


Figure A3. 4: Absolute humidity in the 1L bags for each substrate and storage temperature

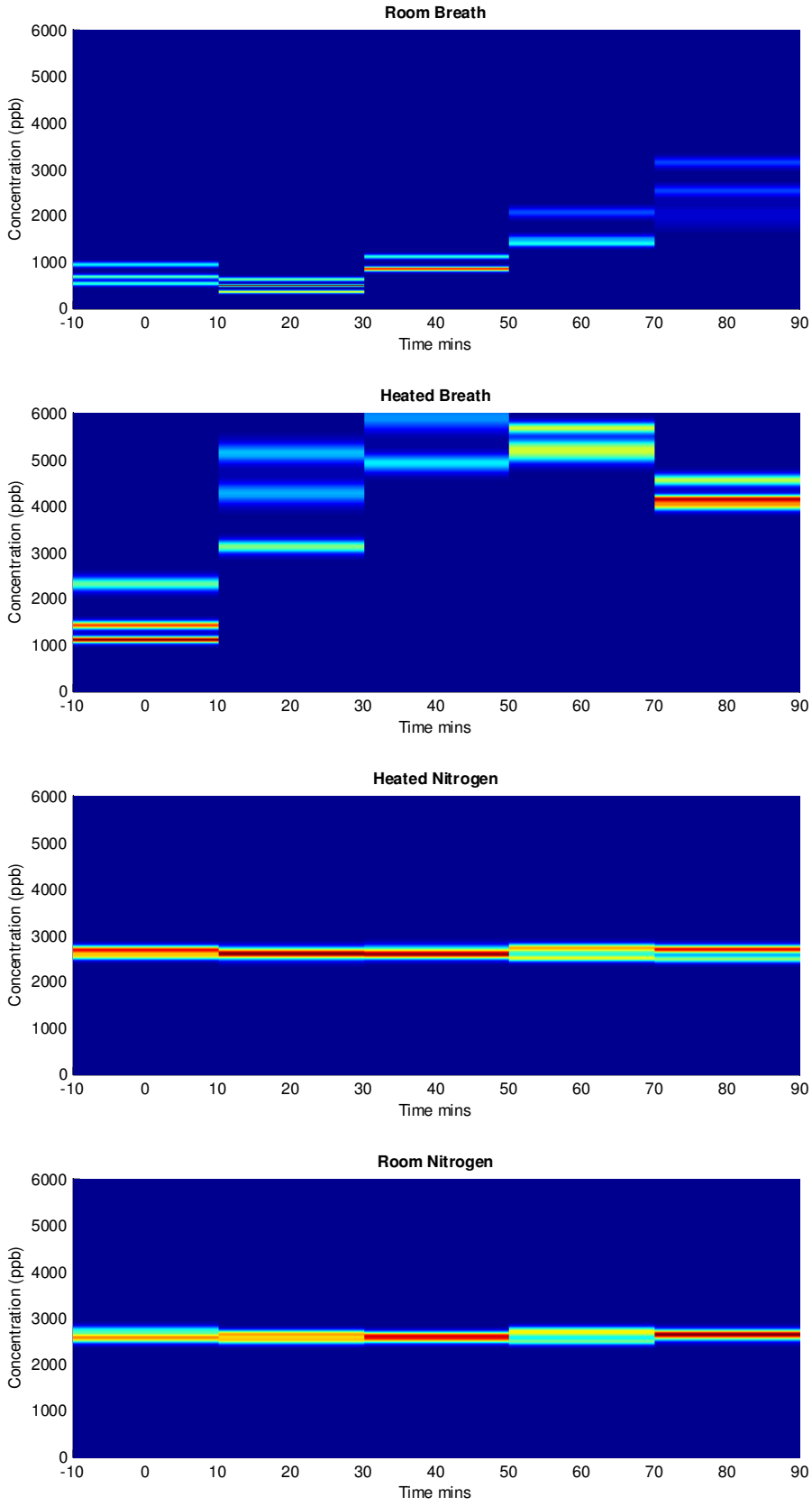


Figure A3. 5: Ammonia monitored using the H_3O^+ precursor in 1L bags test set 2

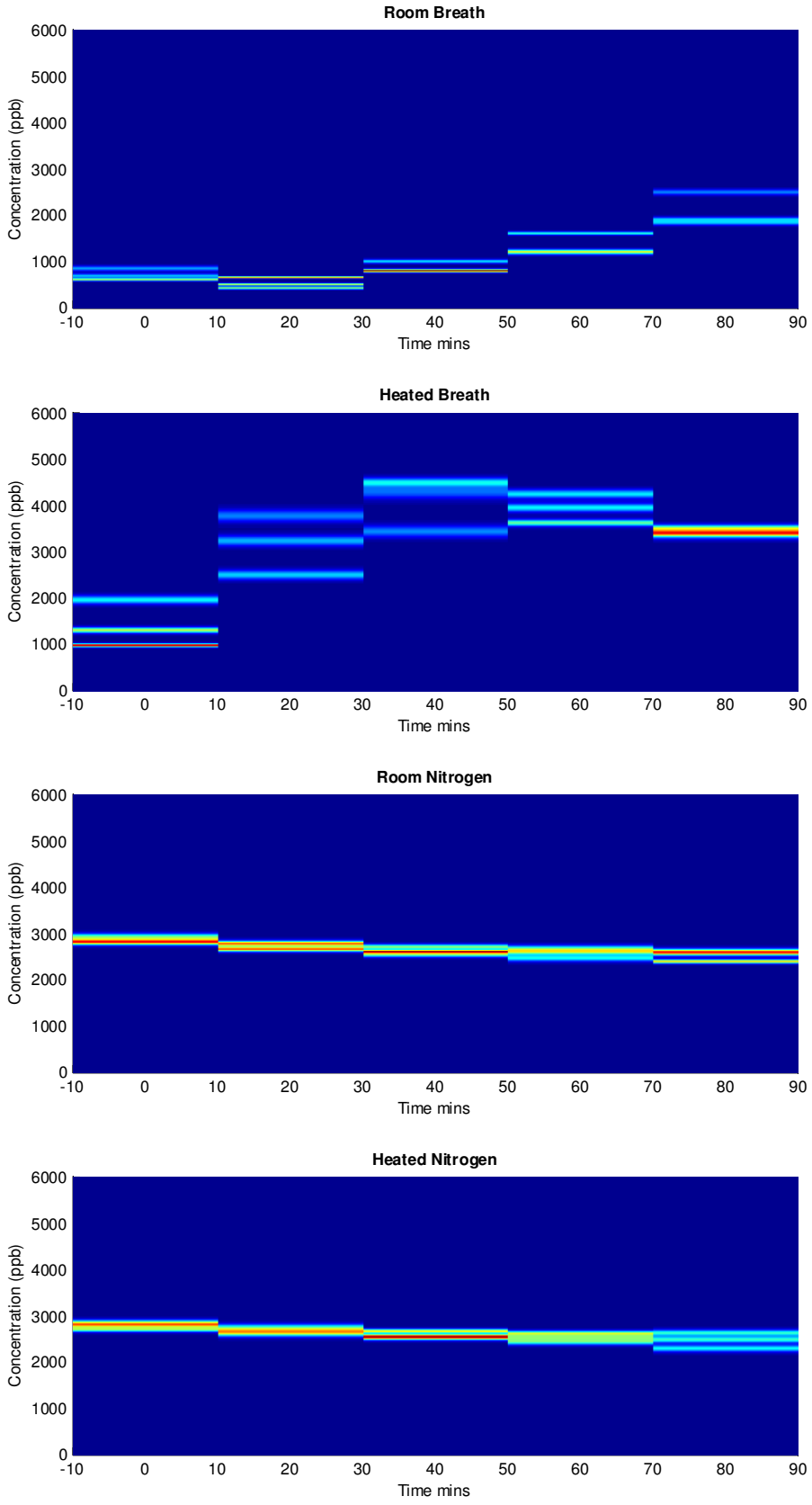
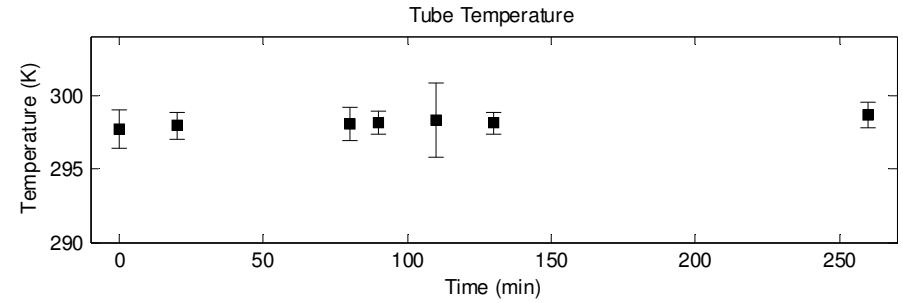
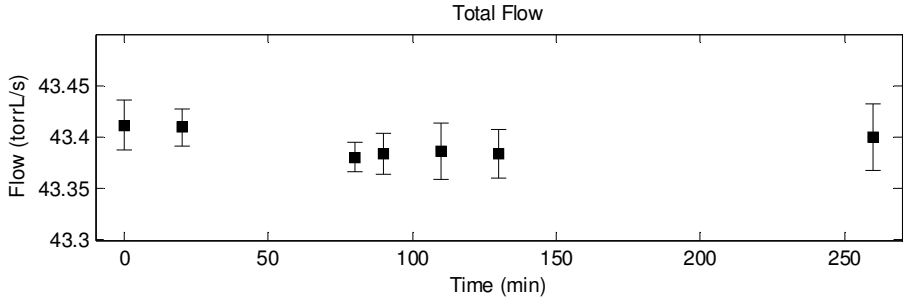
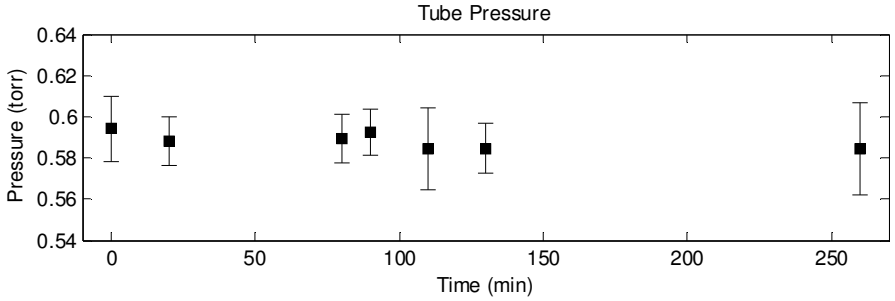


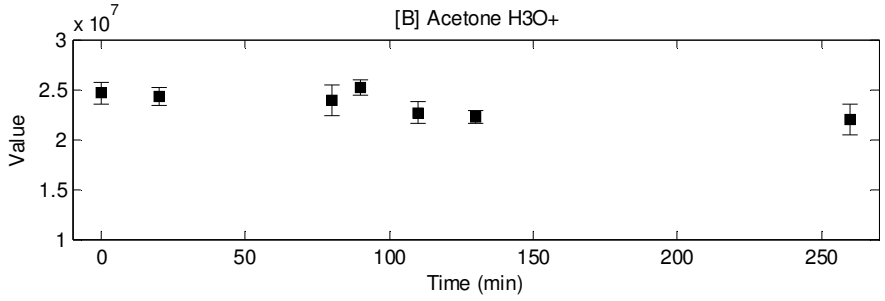
Figure A3. 6: Ammonia monitored using the O_2^+ precursor in 1L bags test set 2

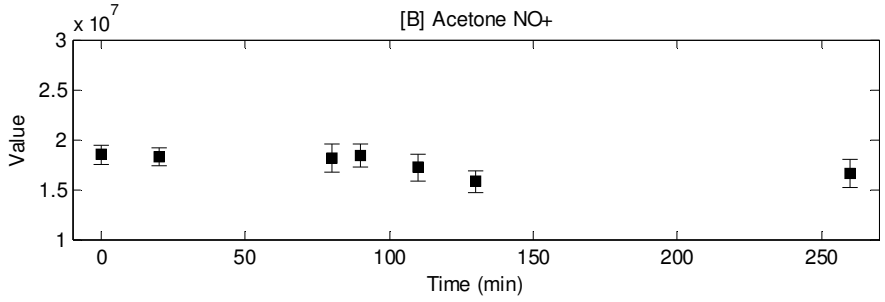
12.4 A4 – Repeatability Tests











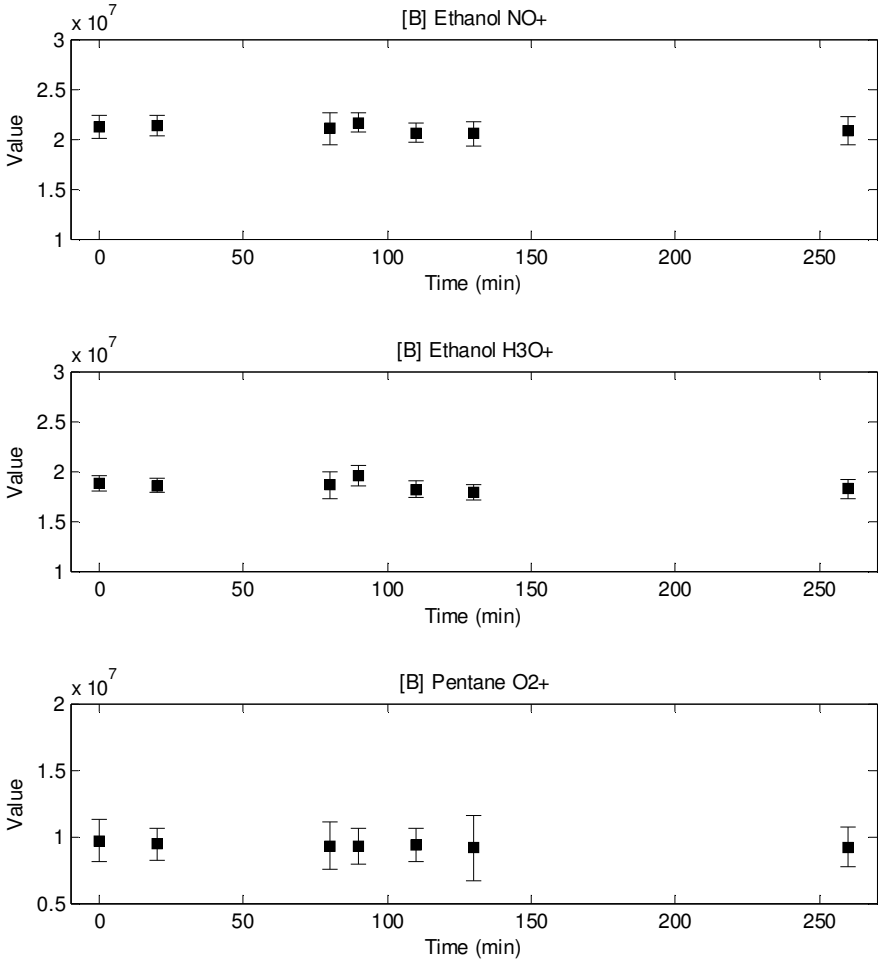


Figure A4. 1: Mean values and including 95% confidence interval for the factors which make up the calculation of the compound concentration

12.5 A5 – Kinetics

ACETONE
H3O+ Reactions
Precursor

H_3O^+	mass 19 k=4.1e-9 col=3.9
$H_3O^+ + H_2O + He \rightarrow H_3O^+.H_2O + He$	mass 37 k=3.0e-9 col=3.2
$H_3O^+.H_2O + H_2O + He \rightarrow H_3O^+.(H_2O)_2 + He$	mass 55 k=2.5e-9 col=2.8
$H_3O^+.(H_2O)_2 + H_2O + He \rightarrow H_3O^+.(H_2O)_3 + He$	mass 73 k=2.2e-9 col=2.4

Products

$H_3O^+ + (CH_3)_2CO \rightarrow (CH_3)_2COH^+ + H_2O$	mass 59
$(CH_3)_2COH^+ + H_2O \rightarrow (CH_3)_2CO.H^+.H_2O$	mass 77 (secoundary) W
$(CH_3)_2CO.H^+.H_2O + H_2O \rightarrow (CH_3)_2CO.H^+.(H_2O)_2$	mass 95 (secoundary)

NO+
Precursor

NO^+	mass 30 k=1.5e-9 kcol=3.3e-9 br=1
$NO^+ + H_2O + He \rightarrow NO^+.H_2O + He$	mass 48 k=0 kcol=3.3e-9

Products

$(CH_3)_2CO + NO^+ \rightarrow NO^+.(CH_3)_2CO$	mass 88

ISOPRENE
O2+
Precursor

O_2^+	mass 32 k=1.3e-9 kcol=1.6e-9

Products

$O_2^+ + C_5H_8 \rightarrow C_5H_8^+ + O_2$	mass 68 br=0.45
$O_2^+ + C_5H_8 \rightarrow C_5H_7^+ + H + O_2$	mass 67 br=0.45
$O_2^+ + C_5H_8 \rightarrow C_4H_5^+ + CH_3 + O_2$	mass 53 br=0.1

H3O+
Precursor

H_3O^+	mass 19 k=2.2e-9 kcol=2e-9
$H_3O^+ + H_2O + He \rightarrow H_3O^+.H_2O + He$	mass 37 k=1.7e-9 kcol=2e-9
$H_3O^+.H_2O + H_2O + He \rightarrow H_3O^+.(H_2O)_2 + He$	mass 55 k=9e-10 kcol=2e-9
$H_3O^+.(H_2O)_2 + H_2O + He \rightarrow H_3O^+.(H_2O)_3 + He$	mass 73 k=8e-10 kcol=2e-9

Products

$H_3O^+ + C_5H_8 \rightarrow C_5H_8.H^+ + H_2O$	mass 69
$C_5H_8.H^+ + H_2O \rightarrow C_5H_8.H^+.H_2O$	mass 87 (secoundary)

NO+

Precursor

NO^+	mass 30 k=1.4e-9 kcol=1.7e-9
$NO^+ + H_2O + He \rightarrow NO^+.H_2O + He$	mass 48 k=1.4e-9 kcol=1.7e-9

Products

$NO^+ + C_5H_8 \rightarrow C_5H_8{}^+ + NO$	mass 68 br=0.9
$H_2O + C_5H_8{}^+ \rightarrow C_5H_8{}^+.H_2O$	mass 86 (secoundary)
$NO^+ + C_5H_8 \rightarrow C_5H_8{}^+.NO$	mass 98 br=0.1

ETHANOL

H3O+

Precursor

H_3O^+	mass = 19 k= 2.6 kcol = 2.7
$H_3O^+ + H_2O + He \rightarrow H_3O^+.H_2O + He$	mass = 37 k=2.6 kcol = 2.7
$H_3O^+.H_2O + H_2O + He \rightarrow H_3O^+.(H_2O)_2 + He$	mass = 55 k = 0.9 kcol = 2.7
$H_3O^+.(H_2O)_2 + H_2O + He \rightarrow H_3O^+.(H_2O)_3 + He$	mass = 73 k = 0.9 kcol = 2.7

Products

$H_3O^+ + C_2H_5OH \rightarrow C_2H_5OH_2{}^+ + H_2O$	mass = 47
$C_2H_5OH_2{}^+ + H_2O \rightarrow C_2H_5OH_2.H_2O$	mass = 65
$C_2H_5OH_2.H_2O + H_2O \rightarrow C_2H_5OH_2.(H_2O)_2$	mass = 83

NO+

Precursor

NO^+	mass 30 k=8.0e-10 kcol=2.3e-9
$NO^+ + H_2O + He \rightarrow NO^+.H_2O + He$	mass 48 k=8.0e-9 kcol=2.3e-9

Products

$NO^+ + C_2H_5OH \rightarrow C_2H_5O^+ + NO$	mass 45
$C_2H_5O^+ + H_2O \rightarrow C_2H_5O^+.H_2O$	mass 63 (secoundary)
$C_2H_5O^+.H_2O + H_2O \rightarrow C_2H_5O^+.(H_2O)_2$	mass 81 (secoundary)
$C_2H_5O^+.(H_2O)_2 + H_2O \rightarrow C_2H_5O^+.(H_2O)_3$	mass 99 (secoundary)

PENTANE
O2+
Precursor

O_2^+	mass 32 k=1.2e-9 kcol=1.6e-9

Products

$O_2^+ + C_5H_{12} \rightarrow C_5H_{11}^+ + O_2 + H$	mass 71
$O_2^+ + C_5H_{12} \rightarrow C_5H_{12}^+ + O_2$	mass 72

AMMONIA
H3O+
Precursor

H_3O^+	mass = 19 k= 1.7 kcol = 2.71
$H_3O^+ + H_2O + He \rightarrow H_3O^+.H_2O + He$	mass = 37 k=1.3 kcol = 2.71
$H_3O^+.H_2O + H_2O + He \rightarrow H_3O^+.(H_2O)_2 + He$	mass = 55 k = 0.88 kcol = 2.71
$H_3O^+.(H_2O)_2 + H_2O + He \rightarrow H_3O^+.(H_2O)_3 + He$	mass = 73 k = 5.5 kcol = 2.71

Products

$H_3O^+ + NH_3 \rightarrow NH_4^+ + H_2O$	mass = 18
$NH_4^+ + H_2O \rightarrow NH_4^+.H_2O$	mass = 36 (secoundary)
$NH_4^+.H_2O + H_2O \rightarrow NH_4^+.(H_2O)_2$	mass =54 (secoundary)

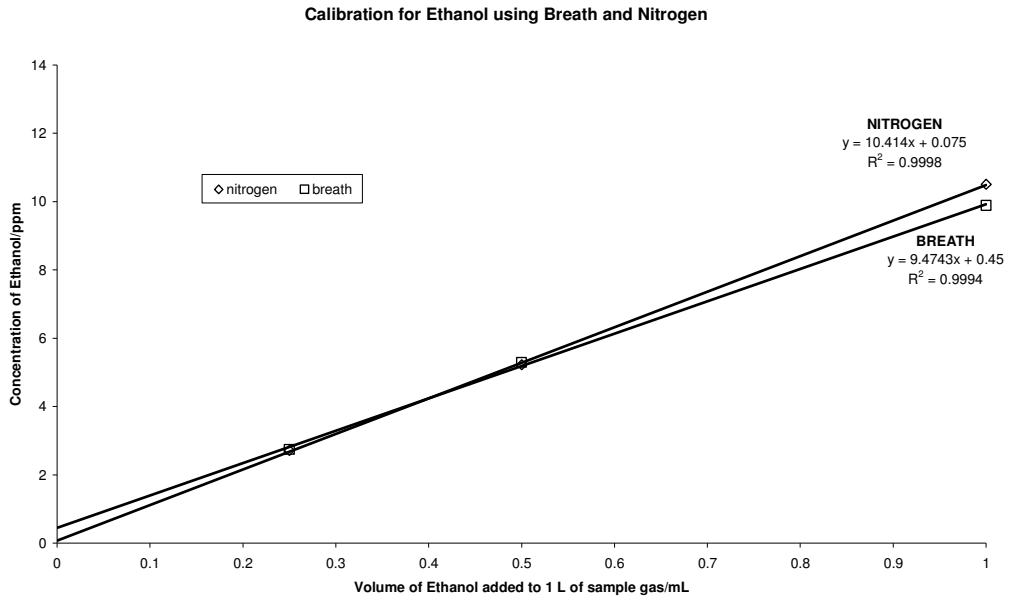
O2+
Precursor

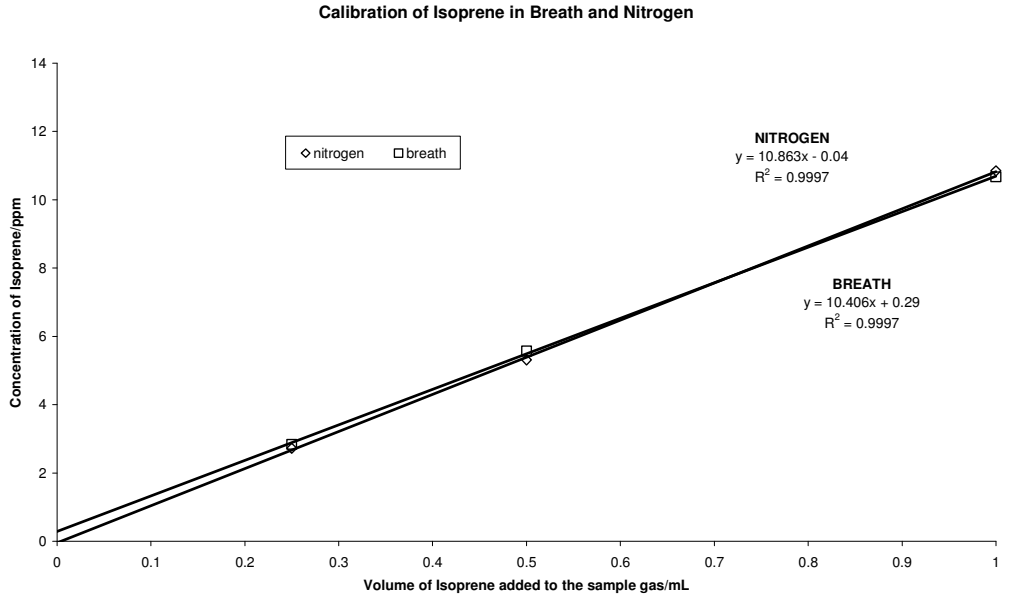
O_2^+	mass 32 k=1.1e-9 kcol=2.4e-9

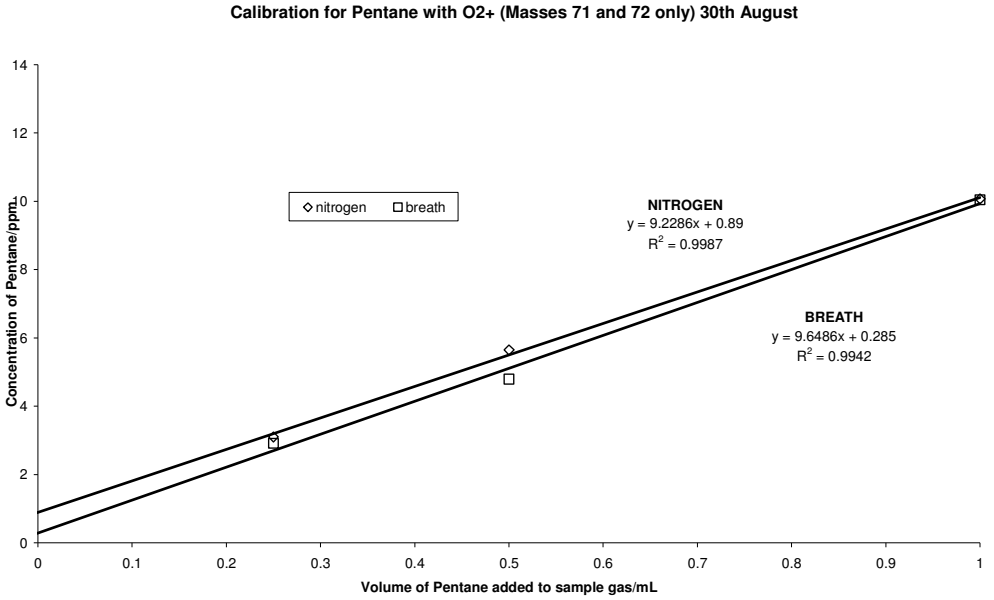
Products

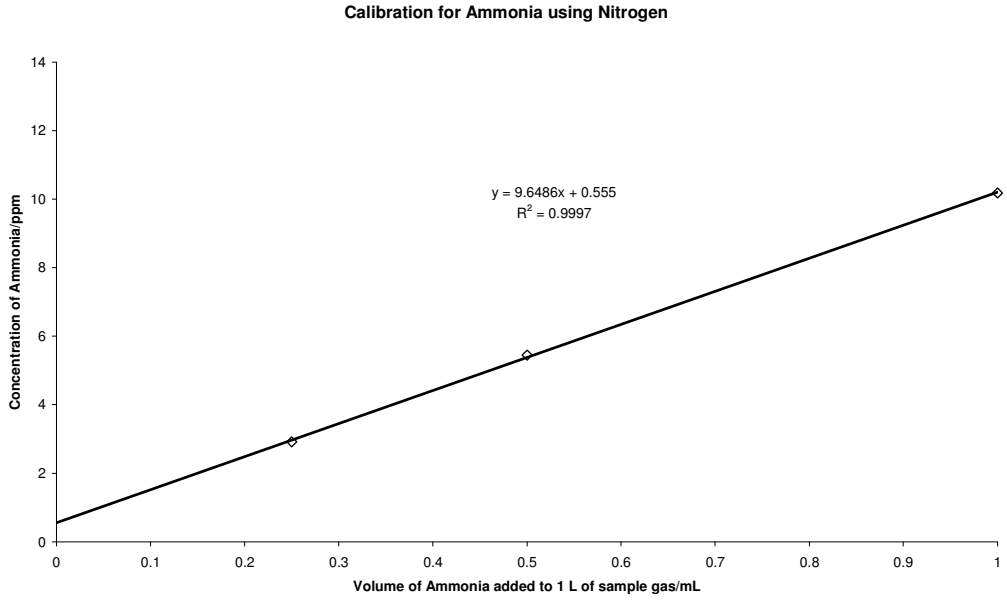
$O_2^+ + NH_3 \rightarrow NH_3^+ + O_2$	mass 17 br=0.7
$NH_3^+ + H_2O \rightarrow NH_3^+.H_2O$	mass 35 (secoundary)
$NH_3^+.H_2O + H_2O \rightarrow NH_3^+.(H_2O)_2$	mass 53 (secoundary)
$O_2^+ + NH_3 \rightarrow NH_4^+ + O_2$	mass 18 br=0.3
$NH_4^+ + H_2O \rightarrow NH_4^+.H_2O$	mass 36 (secoundary)
$NH_4^+.H_2O + H_2O \rightarrow NH_4^+.(H_2O)_2$	mass 54 (secoundary)

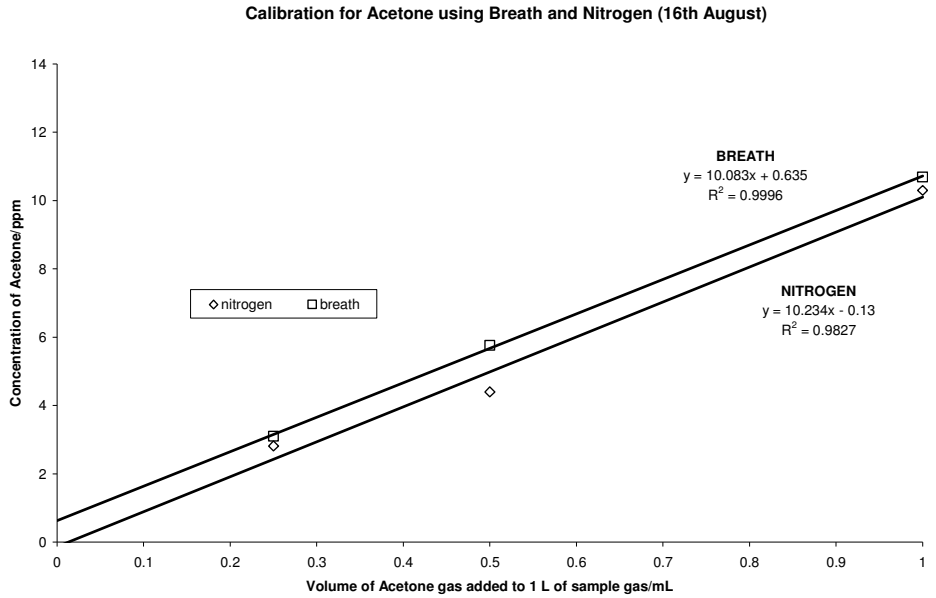
12.6 A6 – Calibration Curves






Calibration for Pentane with O2+ (Masses 71 and 72 only) 30th August
nitrogen
breath
NITROGEN
y = 9.2286x + 0.89
R2 = 0.9987
BREATH
y = 9.6486x + 0.285
R2 = 0.9942
Concentration of Pentane/ppmL
Volume of Pentane added to sample gas/mL
0
2
4
6
8
10
12
14
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1


Calibration for Ammonia using Nitrogen
y = 9.6486x + 0.555
R2 = 0.9997
Concentration of Ammonia/ppm
Volume of Ammonia added to 1 L of sample gas/mL
0
2
4
6
8
10
12
14
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1


Calibration for Acetone using Breath and Nitrogen (16th August)
Concentration of Acetone/ppm
Volume of Acetone gas added to 1 L of sample gas/mL
nitrogen
breath
BREATH
y = 10.083x + 0.635
R2 = 0.9996
NITROGEN
y = 10.234x - 0.13
R2 = 0.9827
0
2
4
6
8
10
12
14
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1